The important role of early diagnosis and preventive management during a large-scale outbreak of hepatitis A in Thailand

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Introduction: Acute hepatitis A is a worldwide public health problem especially in developing countries. Recently, a large, community-wide outbreak of hepatitis A occurred in the northeast part of Thailand.

Methods: Demographic and clinical data as well as blood samples were collected and analyzed from patients with acute hepatitis who attended the Buengkan Provincial Hospital from June to September 2012. About 1619 patients with clinical symptoms of hepatitis A visited the hospital during the outbreak which manifested in three waves. Blood samples were collected from 205 patients.

Results: One hundred and seventy eight patients had hepatitis A confirmed by the presence of anti-hepatitis A virus (HAV) IgM and/or HAV-RNA. The sensitivities for anti-HAV IgM and HAV-RNA were 95.5% (170/178) and 61.8% (110/178), respectively. When HAV-RNA was combined with anti-HAV IgM test, this increased the diagnostic yield by 7.2% (8/111) in the early phase of the acute infection (less than 5 days). Investigation of the molecular structure of the detected viruses indicated that all of the infections were caused by HAV genotype IA. There were no fatalities from this outbreak. Rapid detection, health education, sanitation campaigns, and vaccination offered on a voluntary basis have steadily reduced the number of infected patients and stopped the outbreak.

Conclusion: Occasionally a large-scale outbreak of HAV genotype IA can occur. A combination of HAV-RNA and anti-HAV IgM tests can increase the diagnostic yield during the early phase of the acute infection. Early diagnosis and preventive management campaigns can slow down and stop the outbreak.

Keywords: Hepatitis A, HAV, Genotype IA, Thailand, Outbreaks

Introduction

Acute hepatitis A continues to be a public health problem in developing countries especially in Asia, Africa, and Latin America. Hepatitis A virus (HAV) is a single-stranded RNA virus from the genus *Hepatovirus* and is a member of the *Picornaviridae* family.¹ There are seven genotypes of HAV (I–VII) which can be easily acquired via the fecal–oral route through contaminated food or water and physical contact with infected individuals.² Some infected adults do not exhibit any symptoms whereas others may experience mild flu-like illness, fulminant hepatitis, or severe acute liver failure.³ Acute HAV is diagnosed by detecting the presence of HAV-specific antibodies and antigens. Molecular epidemiology

studies can identify the strain/genotype, extent, route of transmission, and source of infection.⁴

In Thailand, before 1981, more than 97% of adults were anti-HAV positive.⁵ This data indicated that HAV was prevalent throughout the country. When campaigns to improve sanitation and hygiene were enforced, this dramatically reduced the number of HAV outbreaks.⁶ But in the last two decades, HAV outbreaks started to emerge again in child care centers, primary school children, and Thai navy recruits.^{7–11} Sporadic and cyclic HAV outbreaks are common however a large-scale outbreak is rare and disconcerting. In 2012, a large, community-wide outbreak of HAV occurred in the Buengkan province (Fig. 1). As a result of this, we took this opportunity to further investigate this outbreak by focusing on the molecular epidemiology and laboratory diagnosis of the infection. The findings from this study will be used to recommend preventive measures for controlling a future large scale outbreak.

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Figure 1 Map of Thailand showing the location of Buengkan province where the large-scale hepatitis A virus (HAV) outbreak occurred in 2012 (*Location Buengkan province).

Material and Methods

Basic demographic, clinical data and blood samples were collected from patients attending the Buengkan Provincial Hospital during the HAV outbreak (June–August 2012). We defined clinical suspicion of acute hepatitis by symptoms of malaise, fever, abdominal discomfort, nausea, vomiting, jaundice, and dark urine. Two hundred five patients with suspected acute hepatitis were randomly selected from the first peak of the outbreak cohort and sent to the Center of Excellence in Clinical Virology, Chulalongkorn University for diagnosis (anti-HAV IgM by ELISA and HAV-RNA by RT-PCR) and HAV molecular characterization.

Outpatients who attended the hospital with symptoms suggestive of acute hepatitis with or without laboratory confirmation were also included. More than 90% of cases were positive anti-HAV IgM by rapid test (Standard Diagnostics INC, Kyonggi, Korea) All of the admitted cases tested positive for anti-HAV-IgM by rapid test, and comprised the cases recruited for this study.

Duration of hospitalization and clinical outcome of the patients were also recorded and analyzed. The director of the Buengkan Provincial Hospital gave the authors permission to conduct this study and

report its findings. The Institutional Review Board, Faculty of Medicine, Chulalongkorn University has exempted this study from review.

Laboratory Methods

Serological test for anti-HAV IgM

The serum specimens were tested for anti-HAV IgM by using ELISA kits (ARCHITECT HAV Ab-IgM, Abbott Laboratories, Wiesbaden, Germany). The cut-off levels for anti-HAV IgM titers were calculated as specified by the manufacturer.

HAV-RNA detection and genotyping

HAV-RNA was extracted from human serum by using Extraction kit (HiYield™ Viral Nucleic Acid extraction kit, Real Biotech Corporation, Taipei, Taiwan) according to the manufacturer's instruction. Next, a total of 10 µl of RNA was converted to cDNA by using a ImProm-II™ Reverse Transcriptase kit (Promega, Madison, WI, USA). Once this was completed, nested PCR was performed on the cDNA using two sets of primers. For the first round of amplification, an outer sense primer BR-5b (nt 2950–2972): 5'-TTG TCT GTC ACA GAA CAA TCAG-3' and an outer antisense primer BR-9b (nt 3310–3286): 5'-AGT.CAC. ACC.TCT.CCA.GGA.AAA.CTT-3' were used. For the second round of amplification, the following primers were used: RJ-3c (nt 2984–3002): 5'-TCC CAG AGC TCCATT GAA-3' and BR-6b (nt 3217–3193): 5'-AGG AGGTGG AAG CAC TTC ATT TGA-3'.¹² For the first and second amplification steps, 2 µl and 0.5 µl of cDNA were used respectively. For both amplification steps, the reaction mixture was composed of template, 10 µl of 2.5-Master Mix (5 prime, Hamburg, Germany), final concentration of 0.5 µM for each primer, and nuclease-free water up to a final volume of 25 µl. Both amplification reactions were carried out in a Mastercycler Personal (Eppendorf, Hamburg, Germany). After an initial denaturation step at 94°C for 3 minutes, amplification was performed for 40 cycles (94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1 minute), followed by a final extension step at 72°C for 7 minutes. The expected amplified product (approximately 234 bp in length) was visualized on a UV transilluminator (Gel Doc 1000; Bio-Rad, Hercules, CA, USA) after electrophoresis on a 2% agarose gel stained with ethidium bromide. The purified DNA served as templates for DNA sequencing performed by First BASE Laboratories Sdn Bhd (Selangor Darul Ehsan, Malaysia). The nucleotide sequences were analyzed in both directions using forward and reverse primers to confirm the consistency of the sequencing result and ensure that variations of nucleotide sequences were not as a result of sequencing errors.

Figure 2 Phylogenetic tree of hepatitis A virus (HAV) strains based on the VP1-2A region of 200 nt sequences which was constructed with a MEGA software version 5.05 using the neighbor-joining method. The samples from this outbreak are indicated at the respective branches with symbol ▲ and samples from previous study are indicated by symbol □. Bootstrap values are indicated for the major nodes of the percentage on 1000 replicates.

BLAST and Phylogenetic analysis

The partial VP1 sequence was compared with the corresponding GenBank reference sequences of genotypes IA, IB, IIB, IIIA, IIIB, IV, V, VI, and VII (Fig. 2). All sequences were multiple aligned using Clustal X Version 1.8 and the nucleotide

identities with reference sequences were analyzed using the BioEdit Sequence Alignment Editor version 5.0.9. Phylogenetic trees were constructed using the neighbor-joining method of the MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 software.

Figure 3 Three waves of acute hepatitis A patients during the epidemic and intervention phase.

The genotype was determined by BLAST/FASTA (<http://www.ncbi.nlm.nih.gov>) and phylogenetic analysis. The sequences were edited and assembled by using programs CHROMAS LITE v.2.0 (www.technelysium.com.au) and SeqMan (DNASTAR, Madison, WI, USA). To investigate the relationships among HAV strains, an un-rooted tree topology based on multiple alignments of the partial VP1-2A and nucleotide sequences of known genotypes from GenBank were established by the neighbor-joining method, calculated with MEGA 3.1 (<http://www.megasoftware.net>). Consistency of branching was tested by bootstrap analysis with 1000 re-samplings of the data using MEGA 3.1. Multiple protein translation and sequence alignments were generated with BioEdit version 7.0.1 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>).

Statistical analysis

Continuous variables were compared among groups using unpaired *t*-test and one-way ANOVA. Categorical variables were compared among groups using chi-square/Fisher's exact test. All statistical analyses were performed using SPSS version 17.0.

Results

Between June and September 2012, there was a large scale outbreak of hepatitis A at Buengkan province, northeast of Thailand. (Fig. 1) One thousand six hundred nineteen patients visited Buengkan Provincial Hospital with clinical symptoms of acute hepatitis.

Figure 4 Results from anti-HAV IgM and HAV-RNA assays when analyzed individually. (Areas highlighted indicate additional acute hepatitis A virus (HAV) cases detected when anti-HAV IgM test was combined with HAV-RNA.)

Three waves of acute hepatitis A were detected in this outbreak (Fig. 3). About 495 patients were hospitalized. The most common symptoms among those hospitalized were febrile (97.4%), icterus (93.5%), and abdominal symptoms (55.8%). During the outbreak, blood samples were randomly collected for molecular investigation from 205 patients with acute hepatitis. There were 126 males and 79 females. The age of the patients ranged from 2 to 70 years with a median age of 22 years. The median lag time between onset of symptoms and hospital visit was 4 days (range: 1–30 days). From 205 patients, only 178 had confirmed diagnosis of acute hepatitis. Anti-HAV IgM and HAV-RNA assays were used to confirm the diagnosis of acute hepatitis which had a sensitivity of 95.5% and 61.8% respectively. Only eight patients had positive HAV-RNA and negative anti-HAV IgM results (Table 1). All of them were in early phase (< 5 days) after the onset of symptoms. Of the 170 patients with positive anti-HAV IgM, 68 cases tested negative for HAV-RNA (Fig. 4). When the HAV-RNA results were combined with anti-HAV IgM, this increased the diagnostic yield by 7.2% in the early phase of acute infection (less than 5 days). When regions from VP1 to P2 were analyzed at the molecular

Table 1 Basic characteristics of the acute hepatitis A patients based on the results from the hepatitis A virus (HAV) PCR and anti-HAV IgM test*

	All cases (n = 178)	Positive serum HAV RNA** (n = 8)	Positive serum anti-HAV IgM (n = 68)	Positive both (n = 102)	P value†
Mean age(years)	23.1±10	26.1±9	23±10.3	23±9.9	0.91
Duration of presentation(days)	4.2±3.8	2.5±1.2	4.3±3.9	4.4±3.9	0.62
TB(mg/dl)	7.8±9	1.3±0.8	6.7±7.2	9±10.1	< 0.01
DB(mg/dl)	6.2±6.9	0.9±0.7	5.5±5.4	7±7.9	< 0.01
AST(U/l)	1602±1509	1171±756	1460±1327	1720±1645	< 0.01
ALT(U/l)	2160±1647	1049±794	2030±1112	2322±1914	< 0.01
ALP(U/LI)	279±127	210±74	275±110	285.9±138.6	0.04

*Plus-minus values are presented as means±SD for all comparisons.

**All of them were in early phase (< first 5 days) after the onset of symptoms.

†Comparison among three groups (Positive Serum HAV RNA, Positive Serum Anti-HAV IgM, and Positive both).

level, it was shown that all HAV infections from this outbreak were genotype IA. (Fig. 2)

The country's response to the outbreak after its detection is shown in Fig. 3. All sectors of the government became involved in providing health education to the community, especially by targeting open-air markets, restaurants, schools, primary day care centers, and water supply departments. Hepatitis A virus was detected early through the combined efforts of the Buengkan Provincial Hospital and all of its local healthcare units within the vicinity of the outbreak. Hepatitis A virus vaccines were made available as immunoprophylaxis to all healthcare workers and offered on a voluntary basis to members of the high-risk population. The number of cases with acute hepatitis A infection continued to decline and no case of acute hepatitis A was seen after the third wave of the outbreak (Fig. 3).

According to the primary investigation conducted by the Ministry of Public Health, the possible source of an outbreak may have come from an ice making factory. Hepatitis A virus RNA was detected in the ice samples.¹³

Discussion

Periodic outbreaks of HAV are commonly found in developing countries with improved living standards. Hepatitis A virus is transmitted by the fecal-oral route and can be easily transported from an endemic area to a non-endemic area owing to wide spread traveling.¹⁴ Patients with hepatitis A super-infection with chronic viral hepatitis B and C have a higher mortality rate.¹⁵ Since Thailand is endemic for HBV and HCV, there were 4–5% HBV carriers and 2% HCV carriers.^{16,17} No fatalities were detected from this large-scale HAV outbreak.

In this large-scale outbreak, most of the cases were young adults and had typical symptoms of acute hepatitis such as febrile, icterus, and abdominal symptoms. Laboratory data showed significantly elevated levels of transaminase and bilirubin. However, in one patient who had a positive serum HAV-RNA and negative HAV IgM result, the level of bilirubin was slightly elevated whereas the transaminase level was a little bit lower than the norm. This may be the result of the patient's poor immune response to HAV. The diagnosis of acute hepatitis A, in general, uses ELISA to detect anti HAV IgM. In this study the sensitivity amounted to 95% compared to serum HAV-RNA (62%). Our study demonstrated that serum HAV-RNA is superior in the early phase of infection or, during the first 5 days after the onset of symptoms. At that time, anti HAV IgM is not detectable in some cases. As for the strain of HAV, all of the patients had genotype IA. This genotype is common in this region and has been the cause of other previous outbreaks in Thailand.^{7,9–11}

A major limitation to controlling this outbreak was the delayed diagnosis of patients. Buengkan is a provincial hospital which lacks the proficiency to diagnose acute HAV infection definitely. After the group of patients from the first wave was admitted, their serum was sent to the Center of Excellence in Clinical Virology in Bangkok for testing anti-HAV IgM and HAV-RNA. The results were then immediately sent back to help manage and control the outbreak. This outbreak was manageable during the second and third waves and eliminated henceforth. A combination of different preventive measures targeting personal hygiene, sanitation, health education, and HAV vaccines helped control the outbreak and prevented the fourth wave from occurring. Early administration of single dose HAV vaccine was successful in containing the infection to a small group of people.¹⁸ Time lapse between diagnosis and intervention is important and has been associated with shorter periods of outbreak, especially for infections that are transmitted easily from person-to-person like HAV.¹⁹ Hepatitis A virus vaccine was not routinely given to the community during the outbreak owing to limited budget but offered as one of the choices for preventive measures.

The benefit of the vaccine can be seen when the universal HAV vaccination program of preadolescents was implemented in Brazil.²⁰ In the long run, this strategy was cost effective by significantly reducing the number of cases with icteric hepatitis, deaths, and short life expectancy to 64, 59, and 62% respectively.²¹ In another study, it was shown that universal HA vaccination provided through the Expanded Program on Immunization (EPI) was able to reduce the incidence of acute hepatitis A.²² The importance of HAV vaccination cannot be disputed. However, in a resource-limited setting, this may not be possible owing to budget restraints.

Nevertheless, the authors would like to point out that inactivated HAV vaccines used as pre-exposure prophylaxis among susceptible individuals were effective and safe.²³ Furthermore, HAV vaccines given as a post-exposure prophylaxis were shown to be effective in preventing HAV infection when administered within 2 weeks after exposure to the virus.²⁴ However, all of this can be avoided if routine vaccination for HAV is implemented in the first place.

In Thailand, outbreak of hepatitis A is uncommon and occurs sporadically as well as cyclically even though sanitation and personal hygiene have improved in the past 30 years. During the recent outbreak in Buengkan province, all HAV infections were caused by HAV genotype IA. This indicated that HAV genotype IA was never eradicated and continued to contribute to other outbreaks albeit not as large as this one. The combined application of

HAV-RNA and anti-HAV IgM test increased the diagnostic yield in the early phase of acute infection.

The seroprevalence of anti-HAV in Thailand has dramatically declined in the last few decades which in turn have increased the susceptible population. Thus, if there is no routine universal hepatitis A vaccination in Thailand, outbreaks undoubtedly will start to emerge again in child care centers, primary school children, and the young adult population. Therefore, we believe all children should be vaccinated in order to prevent future outbreaks.

In conclusion, preventive measures were very important in slowing down the outbreak so that the health care centers could cope with the large number of patients. However it should be emphasized that preventive measures should not be limited to outbreaks but should continue regularly. As this is a contagious disease that can be acquired via person-to-person contact, the community should participate in preventing such incidence from occurring again. A routine hepatitis A vaccination program should be adopted and incorporated into the national program to prevent large-scale outbreaks. The findings from this study should be used as recommendations for preventive measures of a future large scale outbreak.

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References

- 1 Martin A, Lemon SM. Hepatitis A virus: from discovery to vaccines. *Hepatology*. 2006;43:S164–72.
- 2 Wheeler C, Vogt TM, Armstrong GL, Vaughan G, Weltman A, Nainan OV, Dato V, *et al*. An outbreak of hepatitis A associated with green onions. *N Engl J Med*. 2005;353:890–7.
- 3 Jeong SH, Lee HS. Hepatitis A: clinical manifestations and management. *Intervirology*. 2010;53:15–9.
- 4 Nainan OV, Xia G, Vaughan G, Margolis HS. Diagnosis of hepatitis a virus infection: a molecular approach. *Clin Microbiol Rev*. 2006;19:63–79.

- 5 Burke DS, Snitbhan R, Johnson DE, Scott RM. Age-specific prevalence of hepatitis A virus antibody in Thailand. *Am J Epidemiol*. 1981;113:245–9.
- 6 Poovorawan Y, Chongsrisawat V, Tangkijvanich P. Problems and prevention of viral hepatitis in Thailand. *J Med Assoc Thai*. 2001;84(Suppl 1):S18–25.
- 7 Poovorawan Y, Theamboonlers A, Chongsrisawat V, Jantaradsamee P, Chutsirimongkol S, Tangkijvanich P. Clinical features and molecular characterization of hepatitis A virus outbreak in a child care center in Thailand. *J Clin Virol*. 2005;32:24–8.
- 8 Sinlaparatsamee S, Nuniem J, Kankao J, Theamboonlers A, Chumdermpadetsuk S, Poovorawan Y. An outbreak of hepatitis A in school children at Nakhon Si Thammarat, southern Thailand. *Southeast Asian J Trop Med Public Health*. 1995;26:104–8.
- 9 Theamboonlers A, Rianthavorn P, Jiamsiri S, Kumthong S, Silaporn P, Thongmee C, *et al*. Molecular characterization of Hepatitis A virus causing an outbreak among Thai navy recruits. *Trop Biomed*. 2009;26:352–9.
- 10 Theamboonlers A, Jantaradsamee P, Chatchatee P, Chongsrisawat V, Mokmala M, Poovorawan Y. Molecular characterization of hepatitis-A-virus infections, in the context of two outbreaks in southern Thailand. *Ann Trop Med Parasitol*. 2002;96:727–34.
- 11 Barameechai K, Sa-Nguanmoo P, Suwannakarn K, Thongmee C, Payungporn S, Chongsrisawat V, *et al*. Molecular characterisation of the hepatitis A virus circulating in the 2001–2005 outbreaks in Thailand. *Ann Trop Med Parasitol*. 2008;102:247–57.
- 12 Bruisten SM, van Steenbergen JE, Pijl AS, Niesters HG, van Doornum GJ, Coutinho RA. Molecular epidemiology of hepatitis A virus in Amsterdam, the Netherlands. *J Med Virol*. 2001;63:88–95.
- 13 Thai Ministry of Public Health. Office of Prevention and Control Disease Weekly Report. <http://dpc11.ddc.moph.go.th/daynews/data/2799.txt>. (accessed 2013 January 8).
- 14 Adamson R, Reddy V, Jones L, Antwi M, Bregman B, Weiss D, *et al*. Epidemiology and burden of hepatitis A, malaria, and typhoid in New York City associated with travel: implications for public health policy. *Am J Public Health*. 2010;100:1249–52.
- 15 Pramoolsinsap C, Poovorawan Y, Hirsch P, Busagorn N, Attamasirikul K. Acute, hepatitis-A super-infection in HBV carriers, or chronic liver disease related to HBV or HCV. *Ann Trop Med Parasitol*. 1999;93:745–51.
- 16 Chimpalree N, Oota S, Phikulsod S, Tangkijvanich P, Poovorawan Y. Hepatitis B and hepatitis C virus in Thai blood donors. *Southeast Asian J Trop Med Public Health*. 2011;42: 609–15.
- 17 Sunanchaikarn S, Theamboonlers A, Chongsrisawat V, Yoocharoen P, Tharmaphornpilas P, Warinsathien P, *et al*. Seroepidemiology and genotypes of hepatitis C virus in Thailand. *Asian Pac J Allergy Immunol*. 2007;25:175–82.
- 18 WHO Position Paper On Hepatitis A Vaccines: June 2012-Recommendations. *Vaccine*. 2013;31:285–6.
- 19 Torner N, Broner S, Martinez A, Tortajada C, Garcia de Olalla P, Barrabeig I, *et al*. Factors associated to duration of hepatitis a outbreaks: implications for control. *PLoS One*. 2012;7:e31339.
- 20 Dominguez A, Oviedo M, Carmona G, Batalla J, Bruguera M, Salleras L, *et al*. Impact and effectiveness of a mass hepatitis A vaccination programme of preadolescents seven years after introduction. *Vaccine*. 2008;26:1737–41.
- 21 Sartori AM, de Soarez PC, Novaes HM, Amaku M, de Azevedo RS, Moreira RC, *et al*. Cost-effectiveness analysis of universal childhood hepatitis A vaccination in Brazil: regional analyses according to the endemic context. *Vaccine*. 2012;30(52):7489–97.
- 22 Craig AS, Schaffner W. Prevention of hepatitis A with the hepatitis A vaccine. *N Engl J Med*. 2004;350:476–81.
- 23 Irving GJ, Holden J, Yang R, Pope D. Hepatitis A immunisation in persons not previously exposed to hepatitis A. *Cochrane Database Syst Rev*. 2012;7:CD009051.
- 24 Victor JC, Monto AS, Surdina TY, Suleimenova SZ, Vaughan G, Nainan OV, *et al*. Hepatitis A vaccine versus immune globulin for postexposure prophylaxis. *N Engl J Med*. 2007;357:1685–94.