Clinical Features and Transmission Pattern of Hepatitis A: An Experience from a Hepatitis A Outbreak Caused by Two Cocirculating Genotypes in Sri Lanka

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Abstract. Sri Lanka is one of the intermediate-endemic areas for hepatitis A virus (HAV), and concerns exist about the increasing HAV-susceptible population. In fact, Sri Lanka recorded a large hepatitis outbreak, possibly hepatitis A, around the end of the Sri Lankan war. It included more than 14,000 patients consisting of local residents, internally displaced personnel, and military personnel in the main combat zone. The outbreak had slowed down by October 2009; however, acute viral hepatitis continued to occur sequentially among military personnel. We obtained clinical information and serum samples from 222 patients with acute hepatitis who visited the Military Hospital Anuradhapura between January and September 2010. Samples were subjected to laboratory testing including HAV-immunoglobulin M and genotyping. Most patients (98.2%) were confirmed as having hepatitis A belonging to two subgenotypes: IA and IIIA. We did not observe any differences in clinical or biochemical features among patients with subgenotypes IA and IIIA except for pale stools and upper abdominal discomfort. During the investigation period, we observed a serial outbreak caused by identical HAV strains with an interval in line with that of typical HAV incubation periods. Most patients in the first outbreak were found in the training center, and patients in the second outbreak were found in multiple places where soldiers were assigned after the training center. These findings indicate that a strain of HAV diffused from one place to another along with movement of infected persons among the HAV-susceptible population. HAV vaccination for high-risk groups, such as young soldiers, is necessary.

INTRODUCTION

Hepatitis A is caused by the hepatitis A virus (HAV), a positive-strand RNA virus belonging to the family Picornaviridae, genus Hepatovirus. Most HAV infections occur through the fecal-oral route, either by direct contact with an infected person or by ingestion of food or water contaminated with HAV. Generally, children have asymptomatic or mild infections, whereas adults with HAV infection are symptomatic. In both symptomatic and asymptomatic cases, seroconversion occurs after infection, and convalescent individuals develop lifelong immunity. HAV strains can be differentiated genetically into six unique genotypes (I–VI), which are defined as groups of viruses with >85% nucleotide sequence identity. Three genotypes (I–III) were detected from human cases and were further subdivided into two subgenotypes (A and B), which differ in sequence in no more than 7.5% of base positions. However, HAV has only one known serotype. Anti-HAV antibody induced by infection or vaccination protects individuals against infection of any strain of HAV.

HAV transmission is changing with the improvement of socioeconomic status, urbanization level, and especially access to clean water and sanitation. The distribution of anti-HAV seroprevalence by age group may reflect the local hepatitis A status. In highly endemic areas, most infections occur in early childhood, and those infected acquire anti-HAV antibodies before the age of 10 years. As infections in early childhood are either asymptomatic or mild, hepatitis A is not a medical problem in these communities. In intermediate or transition areas, HAV circulation is partially reduced by improving hygiene conditions in urban areas, and the susceptible population that does not have anti-HAV antibody grows among the younger population living in urban areas. Along with the traffic between urban and rural areas, or after a drastic social change, HAV is brought into an HAV-susceptible population. Outbreaks of hepatitis A are often reported and recognized as serious public health problems. In low-endemic areas, most cases are sporadic infections or small outbreaks. HAV-susceptible individuals are exposed to infections, mostly limited to specific risk groups, such as children day-care providers, hospital workers, or family members who have direct patient contact, travelers to endemic areas, and men who have sex with men.

According to the HAV-seroprevalence survey performed in urban areas in Colombo, Sri Lanka, in 1975, seroprevalence in the group aged 0–9 years was 91.8%. Another survey suggested that the HAV seroprevalence in the population aged 0–10 years was 38.7% and 11.6% in 1976 and 2001–2002, respectively. HAV incidence among young populations has decreased year by year, whereas simultaneously, the number of HAV-susceptible individuals has increased. Sri Lanka is an intermediate-endemic area. Thus, it is possible that the damage caused by HAV infection in Sri Lanka could be serious.

In fact, Sri Lanka recorded one of the largest viral hepatitis outbreaks around the end of the ethnic conflict between the Government of Sri Lanka and the Liberation Tigers of Tamil Eelam (LTTE) in 2009. It included more than 14,000 patients consisting of local residents, internally displaced personnel (IDP), and military personnel in the Vanni region of Sri Lanka, which was the main combat zone during the Sri Lankan war. The outbreak among residents and IDP had slowed down by October 2009. However, acute viral hepatitis continued to occur sequentially among military personnel. We obtained serum samples from among military personnel patients from...
January to September 2010. Laboratory confirmation and genotyping were performed for all samples, and we confirmed that it was a hepatitis A outbreak and two HAV genotypes were cocirculating among patients: subgenotype IA and subgenotype IIIA. Herein, we describe the data of our investigation to compare the clinical features and biochemical profiles of the two subgenotypes. Also, we indicate a typical HAV-transmission pattern observed among military personnel just after the conflict. This is the first report of hepatitis A genotyping from Sri Lanka.

MATERIALS AND METHODS

During January through September 2010 we carried out a study on patients with clinically suspected HAV infection admitted to the Military Hospital Anuradhapura (MHA), Sri Lanka, which is the main military base hospital that treats military personnel from the Vanni region. This study protocol was approved by the Ethics Review Committee of the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka, and at the National Institute of Infectious Diseases, Japan.

Patients and data collection. All patients who visited the MHA with clinical features suggestive of HAV infection between January 11 and September 23, 2010, were enrolled in this study (Figure 1). All patients were male army personnel. After obtaining written informed consent, clinical features and blood biochemistry on admission and during hospital stay were documented. Ten milliliters of blood were obtained from all patients on admission to the MHA. Separated serum samples were stored at −20°C for HAV serological and molecular tests. During an interview by a doctor, every patient listed his symptoms as well as his medical history, the last three places visited before admission to the hospital, and the length of his stay in each place. Detailed assignment records were not shown, as they contained strategically sensitive information.

Diagnosis. All samples were tested for anti-HAV antibodies. Anti-HAV antibodies, including immunoglobulin G (IgG), M (IgM), and A (IgA) were detected by the inhibition enzyme-linked immunosorbent assay (ELISA) as previously described. Anti-HAV IgM was detected by the IgM-capture ELISA as described previously. Molecular diagnosis was performed by reverse transcription polymerase chain reaction (RT-PCR) using a procedure previously described. Each case of HAV in this investigation was defined as a person who had symptoms compatible with acute viral hepatitis A (e.g., icterus, dark urine, anorexia, and malaise) and was positive for anti-HAV IgM and/or the HAV genome.

HAV genotyping. One hundred and forty microliters of each serum sample was subjected to RNA extraction that was performed by using a QIAamp viral RNA kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Reverse transcription polymerase chain reaction (RT-PCR) and following sequence detected the presence of HAV based on amplification of the VP1/2A region of the HAV genome as previously described.

HAV RNA-positive samples were further analyzed to determine the HAV genotype by using the phylogenetic tree of the VP1/2A junction region. Reference sequences were retrieved from the GenBank database. The reference sequences in this study were subgenotype IA; GBM/WT (accession no. X75215) and subgenotype IIIA; PN_IND/India (accession no. EU011791). CLUSTAL X software (www.clustal.org) was used for alignments. Phylogenetic trees were constructed by using the neighbor-joining method from a Kimura two-parameter distance matrix, and bootstrap resampling was performed 1,000 times. Molecular Evolutionary Genetics Analysis software program was used for this analysis.

Geographical distribution of HAV. We applied a unique method for estimating “the representative infection area” to trace the distribution of HAV. We divided the Vanni region into 10 areas referring to the military operational places. These areas were named “Area 1”–“Area 10.” These 10 areas and three training centers, in total 13 places, were used to trace the geographical distribution of HAV (Figure 2). We assigned the representative infection areas where patients

Figure 1. The trend of number of hepatitis A patients who visited to the Military Hospital Anuradhapura during investigation period.
probably got the HAV infection, based on their interviews. Every patient declared the places where he had worked in the 60 days before hospital admission, and the length of each stay at each place (e.g., 50 days in Area 1, 3 days in Area 2, and 7 days in a nonsubjected area such as his hometown). The place where the patient had stayed for the longest time, with a minimum of 30 days, was considered to represent the place he got infected with HAV in consideration of the incubation period (generally 30 days) for hepatitis A. The patients that were supposed to have acquired the HAV infection out of the Vanni region were eliminated from this analysis.

Statistical analysis. The $\chi^2$ test was used to evaluate differences in inspection items. The statistical test for clinical features and blood chemistries was performed using SPSS Statistics for Windows version 17.0 (SPSS Inc., Chicago, IL). The statistical test for epidemiology was performed by using Statcel version 2 (OMS Publishing Inc., Saitama, Japan). A $P$ value of < 0.05 was considered statistically significant.

RESULTS

Diagnosis of hepatitis A. A total of 222 suspected patients with infective hepatitis were recruited from January to September 2010 in this investigation. Of them, anti-HAV IgM was detected in 214 (96.4%) patients (Table 1). PCR was done for 212 patients, and of them, 167 (78.8%) were positive for HAV RNA. There were four patients that were negative for anti-HAV IgM and HAV RNA. Altogether, 218 (98.2%) patients were confirmed as having acute HAV infection. Mean age of the confirmed patients with HAV infection was 22.9 years (standard deviation, $[SD] = 4.3$ years). Among 148 patients, duration of service in the Army was less than 2 years. Almost all patients were previously healthy, with only five patients revealing a history of a significant medical disorder. These five patients included three patients with bronchial asthma and two patients with epilepsy.

HAV genotyping. Of 167 samples that were positive for HAV RNA, 164 PCR products could be used for sequencing. The subgenotypes that were identified with sequencing were IA ($N = 116, 70.7\%$) and IIIA ($N = 48, 29.3\%$) (Table 2). Subgenotype IA was divided into four clusters, designated IIA1 (51 samples), IIA2 (30 samples), IIA3 (34 samples), and IIA4 (one sample). Subgenotype IIIA was divided into three clusters, designated IIIA1 (40 samples), IIIA2 (five samples), and IIIA3 (three samples) (Figure 3).

Clinical features and blood biochemistry on admission. Fever, malaise, and anorexia were reported as the three leading initial symptoms at onset of disease. On hospital admission, icterus, dark urine, anorexia, and malaise were experienced by more than 90% of patients (Table 2). Blood biochemistry had a wide variation on admission (Table 2). All patients had elevated total bilirubin and liver transaminases on admission.

**TABLE 1**

<table>
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<tr>
<th>Laboratory diagnosis of hepatitis A patients</th>
<th>Anti-HAV IgM</th>
</tr>
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<tbody>
<tr>
<td>PCR Positive</td>
<td>163</td>
</tr>
<tr>
<td>PCR Negative</td>
<td>49</td>
</tr>
<tr>
<td>PCR Not tested*</td>
<td>2</td>
</tr>
</tbody>
</table>

HAV = hepatitis A virus; IgM = immunoglobulin M; PCR = polymerase chain reaction.
* Two samples were not tested using PCR due to insufficient volume.
The mean peak total bilirubin value on admission was 6.6 mg/dL (SD 3.6), and 18% of patients had peak total bilirubin value more than 10 mg/dL. The mean peak alanine aminotransferase (ALT) on admission was 507 and 10.1% had ALT values more than 1,000 IU/L. However, only 22% had elevated serum alkaline phosphatase during hospital admission, and almost all had normal white blood cell (WBC) and platelet counts. None of these patients developed fulminant hepatitis, hepatic encephalopathy, acute liver failure, acute renal failure, hemolytic anemia, or myocarditis during the hospital stay. We had one prolonged cholestasis patient and one recurrent hepatitis patient. Both of them were infected with subgenotype IA.

Analysis of clinical features and blood biochemistry on admission according to genotype IA and IIIA did not show any significant difference except for upper abdominal discomfort and pale stools (Table 2). Also, we confirmed that there was no difference of prothrombin time (PT) by subgenotype, even though we could not check PT in all patients due to lack of facilities.

**Geographical distribution of HAV.** According to the patients’ interviews, we could assign the representative infection areas for 156 patients (Table 3). Most of the patients stayed at one location for more than 30 days before onset. This facilitated the assignment of their representative infection areas. The representative infection areas were distributed in all 10 areas in Vanni region, and the three training centers. Subgenotype IA and IIIA were cocirculating among patients. The cluster “IA1” caused two outbreaks, the first one was between February 20 and 29, and the second one was between March 22 and April 10 (Table 3). In the first outbreak, six of eight patients were potentially infected with HAV in training center-1 (Figure 4). The second outbreak occurred mostly 1 month later after the first outbreak. In the second outbreak, 20 cases were detected. These patients were distributed in eight areas in the Vanni region and at the training center-1. From June 2 to 21, 36 patients visited the MHA (Table 3). Of them, 20 and 10 patients potentially got infected in the training center-2 and Area 9, respectively. Detected clusters were multiple, such as, IA1, IA2, IA3, IIIA1, and IIIA3.

**DISCUSSION**

Sri Lanka is an HAV intermediate-endemic area based on a seroprevalence study conducted in 2001–2002. As with other intermediate-endemic areas, Sri Lanka has a concern about the increasing HAV-susceptible population. The greater the susceptible population, the larger the health damage that may be caused by HAV.

From 1983 to 2009, there had been an on-and-off war against the government by the LTTE in the north and east of the island, mainly the Vanni region. In May 2009, the war ended; concurrently a sharp increase in the number of acute hepatitis cases from the armed conflict area, including residents in Vanni region, persons in IDP camps, and military personnel, was reported. Clinical observation indicated that patients might have been infected with HAV; however, it was impossible to confirm the cases with blood tests due to the security situation. The number of cases among residents and IDP returned to baseline levels (a few cases/week) by October 2009; however, the number of patients among military personnel remained higher than usual.
During our investigation, from January to September in 2010, a constant number of patients belonging to the military visited the MHA, and we recruited 222 patients to this investigation. This investigation had some limitations, for example, patients who visited MHA were only military personnel, mainly young soldiers. In addition, information including strategically sensitive matter, such as the date and places of dispatch in detail, were not disclosed. However, through this investigation, we could obtain interesting observations about hepatitis A. As shown in Table 1, 218 of 222 patients (98.2%) were confirmed to have HAV infection by serological and/or PCR tests. All patients were male, and their mean age was 22.9 (±4.9 years). Only 11 patients were older than 30 years, speculating a high percentage of HAV-susceptible population younger than 30 years. The symptoms among patients in our investigation and the preceding hepatitis outbreak were so compatible that we can confirm that the massive hepatitis outbreak in Sri Lanka in 2009 with nearly 14,000 individuals was actually due to hepatitis A infection.

Of the 218 HAV-positive samples, 164 samples could undergo genotyping. They were classified into two HAV subgenotypes: IA and IIIA. Table 3 shows the trend in the frequencies of HAV clusters among patients by 10-day intervals, except for April 21–May 1 and September 20–23.

![Figure 3. Neighbor-joining phylogenetic tree of the nucleotide sequences of the VP1/2A junction region from the representative hepatitis A virus strains in this study. Reference sequences, subgenotype IA; GBM/WT (accession no. X75215) and subgenotype IIIA; PN_IND/India (accession no. EU011791) were retrieved from GenBank database. The scale bar indicates nucleotide distance.](image)

![Figure 4. The first and second outbreaks caused by cluster IA1.](image)

### Table 3

<table>
<thead>
<tr>
<th>Cluster</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
</tr>
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<tbody>
<tr>
<td>IA1</td>
<td>11</td>
<td>21</td>
<td>31</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>IA2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IA3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IIIA1</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>5</td>
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<tr>
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<td>2</td>
<td>1</td>
</tr>
<tr>
<td>IIIA3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>8</td>
<td>5</td>
<td>7</td>
<td>10</td>
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</table>

<table>
<thead>
<tr>
<th>Cluster</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA1</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>IA2</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IA3</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>IIIA1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IIIA2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IIIA3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Total</td>
<td>18†</td>
<td>18‡</td>
<td>2</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

*The first outbreak caused by HAV-cluster IA1.
†The second outbreak caused by HAV-cluster IA1.
‡The outbreak caused by multiple clusters mainly in the training center-2.
subgenotypes: IA (116 patients) and IIIA (48 patients). Subgenotype IA and IIIA were cocirculating during the investigation period. The predominant subgenotype had shifted from subgenotype IIIA to IA. The only previous documentation of hepatitis A genotyping of Sri Lanka-derived strain was detection of subgenotype IIIA from a patient who returned to Sweden from Sri Lanka in 1984.20 Subgenotype IIIA had been reported as the major HAV genotype circulating in India. A study conducted in the Northern part of India in 2002–2003 revealed cocirculation of HAV subgenotype IA and IIIA.23 Our research area, the Vanni region, is separated from south India by a very narrow stretch of sea called Palk Strait. As there is frequent illegal traveling between Sri Lanka and India, there can be similarities in circulating genotypes of HAV in these two countries, as observed here.

It is a concern whether genotype contributes to the severity of hepatitis A. In this study, we showed that there was no difference in disease severity by HAV subgenotypes IA and IIIA, at least among healthy young males. We did not observe any differences in clinical or biochemical features among subgenotypes IA and IIIA except for pale stools and upper abdominal discomfort. The bilirubin-related markers, except for pale stools, had no difference by genotype. Upper abdominal pain is a subjective symptom, and it is controversial whether to use it as a parameter of disease severity. A study conducted in Korea revealed recent changes in circulating genotypes from IA to IIIA.24 Furthermore, it revealed increased frequency of fever, dark urine, higher aspartate aminotransferase levels, ALT levels, and PT on admission among patients with subgenotype IIIA than patients with subgenotype IA. However, in the same study, it was noted that there was no difference in either peak level of liver transaminases and PT or overall clinical outcome between the two genotypes. In another study conducted in Korea, it was found that age ≥ 40 years, female gender, hepatitis B surface antigen positivity, peak PT, and peak total bilirubin are associated with severe complications such as prolonged cholestasis, acute kidney injury, and acute liver failure and the complication rates were 4.6%, 2.3%, and 2.2%, respectively.25 On the other hand, only one patient in our study had prolonged cholestasis and none had acute kidney injury, acute liver failure, or hepatic encephalopathy. Hematological manifestations of HAV infection include leukopenia and thrombocytopenia. In fact, during a recent outbreak of HAV in Korea, approximately one-third of patients had leukopenia and thrombocytopenia.26 However, in our study, almost all had normal WBC and platelet counts. Thus, genotype could not be the main reason for the differences in clinical features. Hepatitis A severity might depend on the patient’s factors.

During the investigation period, we found a serial outbreak caused by single HAV strain. From February 20 to March 1, during the first outbreak, eight patients visited the MHA with IA1. Of them, six patients possibly got infected at the training center-1. In the second outbreak, from March 22 to April 10, 20 patients were confirmed to have IA1 infection. Their representative infection areas were dispersed into eight areas (19 patients) and the training center-1 (one patient). The interval between the first and the second outbreaks matched the standard incubation period of HAV. The nucleotide sequences of the isolates in the first and the second outbreaks were mostly the same, belonging to IA1 cluster. The strain might have diffused in the training center-1 during the first outbreak, and then asymptomatic patients, carrying HAV from training center-1, would leave for their new posts in the combat zone. The individual developed and/or spread HAV in its new place, and the second outbreak occurred in multiple locations.

From June 2 to 21, 36 patients visited the MHA. Most of the patients were possibly infected in the training center-2 (20 patients) and Area 9 (10 patients). There was no accumulation of specific cluster. It was presumed that all clusters were mixed and endemic enough to cause small outbreaks in multiple places due to the lack of appropriate disease control solutions, such as keeping environmental sanitation, isolation of patients, and immediate vaccination for the susceptible population.

In Sri Lanka, improvement of public health and increasing standards of living are leading to a decreasing incidence of hepatitis. Nevertheless, since the infiltration of Sri Lankan military into the LTTE-occupied area, the number of patients with hepatitis A has gradually increased.15 It is speculated that the ex-LTTE-occupied area was HAV endemic due to insufficient sanitary conditions. Young soldiers, residents, and IDP who were susceptible to HAV were exposed to HAV in the combat zone. Social conditions are closely associated with HAV prevalence, especially in HAV intermediate-endemic areas. In this Sri Lanka case, the drastic change of social conditions, that is, conflict by war, caused a severe public health problem due to hepatitis A. In chaotic social situations, hepatitis A vaccination for high-risk groups, such as young military personnel, and individuals who visit such areas as voluntary support staff from low HAV–endemic areas, is important to reduce the burden of medical expenses and social health issues.

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