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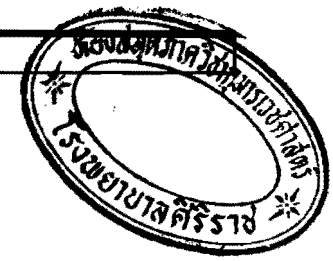

Herpes Simplex Virus Type-2, Cytomegalovirus and Epstein-Barr Virus Infection in Acute Non A to E Hepatitis Thai Patients

Yaowapa Maneerat, Polrat Wilairatana, Emsri Pongponratn, Pilaipan Puthavathana[1], Urai Chaisri, Sucha Kurathong[2], Anuchit Juthaputhi[3], Edward T. Clayson[4], Rapin Snitbhan[4], Boonyos Raengsakulrach[4], and David W. Vaughn[4]

SUMMARY **A significant number of acute non A to E hepatitis cases are reported in Thailand every year, and the etiologies of these cases are unknown. Members of the herpesviridae family have been reported to cause either a self limited or fatal hepatitis in a small proportion of patients in other parts of the world. To determine whether herpesviruses may play a role in acute non A to E hepatitis, sera from 32 acute hepatitis patients without markers for acute hepatitis A to E virus infection were examined for IgM to herpesvirus type 2 (HSV-2), cytomegalovirus (CMV), and Epstein-Barr virus (EBV) using commercially available assays. IgM to HSV-2 was detected in four sera, IgM to CMV was detected in one serum, and IgM to EBV was detected in one serum. All of the acute non A to E hepatitis patients recovered and none had underlying conditions associated with impaired immunity. These results suggest that herpesviruses should be considered in the differential diagnosis for Thai patients with hepatitis.**

Hepatitis is a public health problem in Thailand. The most important etiologies are hepatitis viruses. Hepatitis A virus (HAV), which causes a self-limited acute hepatitis, was once a major problem all over Thailand. Recent improvements in sanitation and in the quality of drinking water have greatly reduced HAV transmission in urban area, whereas HAV transmission in rural areas still results in significant sporadic disease as well as larger outbreaks.[1,2] Hepatitis B virus (HBV) is the leading cause of chronic liver disease in Thailand accounting for nearly 60% of all chronic hepatitis. Hepatitis C virus (HCV) is another cause of chronic liver disease in Thailand accounting for about 35% of all chronic hepatitis. Hepatitis D virus (HDV) requires co-infection with HBV and is rarely observed in Thailand.[3] Hepatitis E virus (HEV) causes a self-limited hepatitis, although often severe in pregnant women and appears to play a small role in Thailand.[4,5] Interestingly, the annual Epidemiology Thai Survey Report, 1992 indicated that about 78% of all cases of hepatitis were uncharacterized.[6] Herpes viruses, including herpes simplex virus (HSV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are responsible for a small proportion of hepatitis world wide.[7-9] The role of HSV-2, CMV and EBV as the causative agents of hepatitis has never reported in Thailand. The purpose of our study was to identify the incidence of herpes viruses in acute non A to E hepatitis in Thailand.

MATERIALS AND METHODS

Study population

Twenty-two control serum samples (8 females and 14 males) were selected from healthy donors

From the Faculty of Tropical Medicine, [1]Faculty of Medicine Siriraj Hospital, [2]Faculty of Medicine Ramathibodi Hospital, Mahidol University; [3]Pramongkutklao Army Hospital; [4]Department of Virology, U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand
Correspondence: Yaowapa Maneerat

at the Blood Bank, Ramathibodi Hospital. Sixty-two study patients consisted of 36 females and 26 males, ages 15-60 years (average 33 years). The sera were collected on the second day of admission from patients clinically suspected acute viral hepatitis admitted to Pramongkutklao Army Hospital, Ramathibodi Hospital, and Hospital for Tropical Diseases, Mahidol University, Bangkok, Thailand from June 1995 to October 1996. Study entry criteria included clinical suspicion of viral hepatitis, elevated alanine aminotransferase (ALT) level (> 45 U/l), and rapid onset of illness. Viral hepatitis was suspected in patients who presented with signs and symptoms that included anorexia, nausea, malaise, abdominal pain, dark urine, jaundice, and sclera icterus. Patients with a history of chronic hepatitis, exposure to hepatotoxic drugs/chemicals, or chronic alcohol use were excluded. The sera were stored at -70°C until analysis.

Laboratory methods

Sera from the patients and controls were examined for anti-HAV IgM, anti HBV core Ag (HBc) IgM and HBV surface Ag (HBsAg) by commercial enzymed-linked immunosorbent assay (ELISA) test kits (HAVAB-M-EIA; AUSZYME-MONOCLONE Test; CORZYME Test, Abbott Laboratories, North Chicago, IL). The sera without markers for acute HAV and HBV infections were further tested for anti-HCV antibody, using a commercial ELISA kit (HCV-EIA 2nd Generation; Abbott Laboratories, North Chicago, IL). The non A, B, C hepatitis sera were further determined for IgM and IgG to HEV using a recently developed ELISA (Genelabs Diagnostics, Singapore).

Using commercial ELISA test kits, sera from the non A, B, C hepatitis cases were also assayed for IgM to HSV-2 (HSV-2 IgM ELISA, Clark Laboratories, Inc., Jamestown, NY), IgG to HSV-2 (HSV-2 IgG ELISA, Clark Laboratories, Inc., Jamestown, NY), IgM to CMV (CMV IgM ELISA, Clark Laboratories, Inc., Jamestown, NY), IgG to CMV (CMV IgG ELISA, Clark Laboratories, Inc., Jamestown, NY), IgM to EBV (EBV IgM ELISA, HUMAN, Germany) and IgG to EBV (EBV IgG ELISA, HUMAN, Germany). The manufacturers' instructions stated that there were no cross reactivity to other herpes common viruses (HSV-1, CMV, EBV, varicella zoster virus), antinuclear antibodies and rheumatoid factor with these kits.

In addition, all sera from cases and control subjects were also assayed for ALT levels to determine the degree of hepatic injury in the patients.

RESULTS

The most common physical findings among patients were jaundice (60%), icterus (60%), and dark urine (50%). Alanine aminotransferase levels in all patients were significantly elevated suggestive of acute viral hepatitis (> 45 U/l). There were no complication nor mortality. There was no evidence to suggest that any of these patients were immune compromised. None of the patients were pregnant, organ recipients, suffering from burns, HIV positive, or undergoing chemotherapy for cancer.

Among the 62 acute hepatitis cases, hepatitis A (anti-HAV IgM positive) was diagnosed in 7 (11.3%), acute hepatitis B (anti-HBc IgM positive) in 16 (25.8%), hepatitis C (total anti-HCV positive) in 5 (8.1%), and hepatitis E (anti-HEV IgM positive) in 2 (6.5%). One of two HEV infected patients had anti-HSV-2 IgM antibody. Total 62 cases, classified according to the causes of hepatitis in proportion of the study, were shown in Table 1.

Thirty-two sera without markers for hepatitis A, B, C, and E were identified for serologic evaluation for hepatitis associated with herpes virus infection. Four of 32 patients (12.5%) had anti-HSV-2 IgM and IgG antibodies, 1 of 32 patients (3.1%) had anti-CMV IgM and IgG antibodies, and 1 of 32 patients had anti-EBV IgM and IgG positive (Table 2). The prevalence of anti-HSV-2 IgG, anti-CMV IgG, and anti-EBV IgG among the 32 acute non A-E hepatitis was 82%, 82%, and 100%, respectively. Among the 22 normal controls, IgM antibody to the herpes viruses was not observed, whereas the prevalence of anti-HSV-2 IgG, anti-CMV IgG, and anti-EBV IgG was 62%, 67%, and 100%, respectively. Twenty-six of acute non A to E hepatitis (81.25%) were uncharacterized in our study (Table 2). The clinical findings of 6 characterized cases of non-A to E hepatitis and one mixed infection of HEV and HSV-2 case are summarized in Table 3.

Table 1. Etiology of hepatitis in the 62 acute hepatitis patients

Suspected etiology of acute hepatitis	Number of patients* (%)
HAV	7 (11.3%)
HBV	16 (25.8%)
HCV	5 (8.1%)
HEV	1 (1.6%)
HSV-2	4 (6.5%)
CMV	1 (1.6%)
EBV	1 (1.6%)
Mixed infection (HEV + HSV-2)	1 (1.6%)
Unidentified cases	25 (40.3%)
Total cases	**62 (100%)**

* Number of patients who had specific IgM to hepatitis viruses or herpes viruses
** Other causes of hepatitis that were not characterized in this study

Table 2. Frequency of HSV-2, CMV and EBV in non A to E hepatitis

Subjects	Number (%) of cases with					
	IgM to			IgG to		
	HSV-2	CMV	EBV	HSV-2	CMV	EBV
Non A to E hepatitis patients (n = 32)	4 (12.5%)	1 (3.1%)	1 (3.1%)	27 (82%)	27 (82%)	32 (100%)
Control (n=22)	0	0	0	14 (62%)	15 (67%)	22 (100%)

Table 3. Clinical findings in the patients with acute markers for infection with herpesviruses

Case No.	Ages (years)	Sex	IgM specific to	Sign			Levels of ALT (IU/l)
				Jaundice	Diarrhoea	Dark urine	
1	32	M	HEV; HSV-2	+	+	+	145
2	41	F	HSV-2	+	-	+	1,050
3	23	M	HSV-2	+	-	+	1,355
4	34	M	HSV-2	+	-	+	190
5	60	F	HSV-2	+	-	+	950
6	40	M	EBV	+	-	+	231
7	24	M	CMV	+	-	+	3,000

DISCUSSION

Herpes viruses search for in our study were HSV-2, CMV and EBV which play roles in causing rare cases of hepatitis in previous studies.[7-13] Herpes viruses cause latent infection and are usually found in most healthy peoples. After first replication, the viruses persist in the nucleus of some host cells without replication nor symptoms.[14] We observed that 100%, 67% and 62% of 22 healthy controls had specific IgG to EBV, CMV and HSV-2, respectively. This finding indicated more than 50% of healthy controls had prior infection or persist infection of the herpes viruses, as seen in other studies.[15] None of the controls had elevated IgM to the herpes viruses (Table 2). The IgM and IgG specific to HSV-2, CMV and EBV in association with the elevation of alanine aminotransferase in sera of some acute hepatitis patients (Table 3) suggest that the herpes viruses were able to cause non A to E hepatitis. However, we can not indicate whether the patients had the primary infection or reactivation of latent infection because the presence of IgG to the specific herpes viruses may be found in both prior infection and primary infection.[16-18] The patients had no complications and fully recovered in 1-2 months.

Four patients with acute non A to E hepatitis had antibody evidence for HSV-2 infection and 1 patient had evidence for a mixed infection of HEV and HSV-2 . All five patients had mild symptoms and no history of underlying diseases, with the ranges of ALT levels from 145-1355 IU/l (Table 3). Contrastly, Chase *et al.*,[7] reported 2 fatal fulminant hepatitis caused by HSV-1. The patients were a 24-year-old woman with a history of systemic lupus erythematosis and a 50-year-old man that had a myelodysplastic disorder as the underlying disease. At necropsy, the livers of both cases showed extensive hemorrhagic necrosis that corresponded to the markly increased ALT levels to 100-1000 times normal.[7] In addition, the most cases of HSV-1 or -2 hepatitis were associated with immunocompromised states related to renal transplantation,[8] pregnancy,[19] severe burn,[20] thymic dysplasia,[21] and the acquired immuno-deficiency syndromes (AIDS).[22, 23]

Although CMV can cause severe neonatal hepatitis[23] or fulminant CMV hepatitis in liver transplanted patients,[24, 25] and patients with AIDS,[11] almost all CMV infection, between the age of 11 and 50 years, are asymptomatic.[26] We found 1 of 32 acute non A to E hepatitis patients with acute markers for CMV infection. This small proportion of CMV hepatitis was also observed previously.[9, 12, 22] This young adult patient, without underlying disease, had IgM specific to CMV and a markly high level of ALT (Table 3). It is possible that the abnormal increase of ALT from severe hepatic injury may be affected by mixed infection of CMV and other microorganisms not identifed in our studies.

Our study revealed one EBV infected hepatitis patient. The patient developed mild symptoms associated with a slight increase of ALT level. EBV hepatitis has been found in previous studies of acute sporadic hepatitis at the same frequency as in our study.[10,12,13] CMV and EBV infection can cause infectious mononucleosis, characterized by presence of atypical large lymphocyte in peripheral blood and inflammation of lymphoid organs.[27,28] Our study showed that EBV and CMV infected patients had no evidence of infectious mononuleosis illness because of their physical examination and normal blood smear.

Severe diseases, EBV lymphoma,[29] CMV pneumonitis,[29] fulminant hepatitis from EBV,[11,30] CMV,[11,31,32] or HSV[7,8] have been observed in immuno-compromised patients (*eg.* those receiving immunosupressive drugs or AIDS patients). But our study indicated that the herpes viruses play roles in small proportion of acute non A to E hepatitis patients without special risk factors. This knowledge should prompt clinicians to consider this small group of herpes viruses in acute hepatitis in Thai patients which can be virulent particularly in AIDS or immunocompromised patients. A specific etiologic diagnosis should suggest an treatment. Specific antibody assay in combination with determination of the genomes of herpes viruses in a larger study population should be conducted to gain better information.

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