

Evaluation of Two Anti-Hepatitis E Virus IgM Kits¹

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Objective To evaluate two commercial anti-hepatitis E virus (HEV) IgM kits used for differential diagnosis of acute enteric viral hepatitis. **Methods** The kit for IgM capture assay, was produced with a recombinant HEV structural protein protecting primates against experimental infection by different HEV genotypes, while the other kit for indirect ELISA was produced with recombinant structural proteins from different HEV genotypes. The serum specimens were taken from 241 cases with a confirmed or presumptive diagnosis of hepatitis A and 74 cases with a confirmed or presumptive diagnosis of hepatitis E. **Results** The sensitivity and specificity of the IgM capture assay kit were 97% and 100%, respectively, and the corresponding values for the other kit were 70% and 78%, respectively. **Conclusion** The IgM capture assay kit has higher sensitivity and specificity in diagnosing acute enteric viral hepatitis E.

Key words: IgM anti-HEV; Viral hepatitis

INTRODUCTION

Hepatitis A virus (HAV) and hepatitis E virus (HEV) are the major pathogens of acute enteric hepatitis. Diseases caused by these viruses have the same patho-physiological process, but are distinct clinical and epidemiologic entities. Humans and possibly non-human primates are natural hosts of HAV, and shell fish is an important conduit of its transmission. Usually, disease caused by the virus is self-limiting, afflicting largely young people in endemic areas and travelers to these areas. HEV infection is zoonotic. The virus is widely distributed among domestic and wild animals worldwide, especially among swine and rodents^[1-5]. Compare with hepatitis A, disease caused by HEV is more severe, and characterized by protracted cholestasis and coagulopathy, afflicting old people in endemic areas in fulminant forms and with high mortality^[6-10]. Both diseases display identical immuno-pathological changes; viral activities usually have subsided and antibody levels have already peaked before the onset of the diseases^[11], which makes the traditional detection of the virus and its antibody level unreliable

for their diagnosis. Therefore, the only appropriate method of diagnosing such conditions is to detect IgM antibodies^[9,12-14].

The present study was to evaluate two immunosorbant assay kits specific for IgM antibody to HEV for differential diagnosis of enteric viral hepatitis. The first kit designated as HEV IgM [E] is a IgM capture assay produced with a recombinant protein spanning amino acid residue 396-607 in the major structural protein of HEV genotype 1. The recombinant protein occurs as a hexamer in neutral solution, which is dissociated into homodimer under denaturing conditions in the presence of 0.1% SDS^[15-16]. The homodimer is a dominant antigenic determinant of the virus^[11,17], including the neutralization sites of the virus^[18]. The recombinant protein protects primates against experimental infection with genotype I or genotype IV HEV strains^[19-20]. The other kit is indirect ELISA designated as HEV IgM [G], which is produced with recombinant structural proteins spanning 327aa residues in the C terminal region of the major HEV structural protein and encompassing major linear epitopes presenting in the viral protein^[21].

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MATERIALS AND METHODS

Patients

Serum samples were obtained from 525 patients admitted to Nanshi Hospital, Shanghai with acute hepatitis between January and December 1992, and kept at -20°C. Two hundred and ten cases were excluded among which 9 cases had a history of alcoholic or drug-induced hepatitis, 72 cases had chronic hepatitis B, 15 cases had acute hepatitis B, 1 case was co-infected with hepatitis B and C, and 113 cases had unknown causes. The remaining 315 were diagnosed as enteric viral hepatitis, positive for IgM anti-HAV and / or IgM anti-HEV by either or both of HEV assays. The diagnosis of enteric hepatitis was supported by exclusion of acute or chronic hepatitis B and non-infectious causes.

IgM Anti-HEV and IgM Anti-HAV ELISA

Samples from the remaining 315 patients were tested by HAV IgM (Abotts Lab, Chicago, USA) and two HEV IgM specific assays, designated as HEV IgM [E] supplied by Wan Tai Pharmaceutic, Beijing, China and HEV IgM [G] supplied by Genelabs, Singapore. Testing was done in duplicates following the

manufacturer's instructions.

RESULTS

The occurrence of HAV and HEV specific IgM antibodies in 315 cases of enteric viral hepatitis is listed in Table 1. These patients were positive for IgM antibody against HAV and/or HEV, negative for IgM anti-HBcAg and IgG anti-HCV. However, they did not have a history of chronic hepatitis B or alcohol or drug-induced hepatitis. One hundred and eighty-seven out of these patients were positive for IgM anti-HAV and negative for anti-HEV IgM and were considered having a confirmed diagnosis of hepatitis A. Another 50 patients were considered having a confirmed diagnosis of hepatitis E, and were positive for both HEV IgM assays and negative for HAV IgM. Six cases were positive for only one HEM IgM, but negative for HAV IgM, and a diagnosis of hepatitis E was thus established. The remaining 72 samples were positive for IgM anti-HAV and one IgM anti-HEV, and co-infection with both viruses was established. However, this diagnosis was not supported by the absence of co-infection where the sample was positive for all the three antibodies.

TABLE 1

Serologic Profile of Sporadic Enteric Hepatitis

Serologic Profile			Diagnosis	Cases (n)
HAV IgM	HEV IgM [E]	HEV IgM [G]		
[+]	[-]	[-]	Confirmed Hepatitis A	187
[-]	[+]	[+]	Confirmed Hepatitis E	50
[-]	[+]	[-]	Presumptive Hepatitis E	4
[-]	[-]	[+]	Presumptive Hepatitis E	2
[+]	[+]	[-]	Confirmed Co-Infection	0
[+]	[+]	[-]	Presumptive Co-Infection	18
[+]	[-]	[+]	Presumptive Co-Infection	54
			Total	315

Note. Serum samples were tested for HAV IgM and anti-HEV IgM with HEV IgM [E] and HEV IgM [G] specific for HEV IgM.

The age distribution of confirmed cases of hepatitis A or E was compared with that of the presumptive cases of co-infection of both viruses (Fig 1). The results showed that confirmed hepatitis A afflicted mainly young individuals. Peak age of infection was 30-39 years and the infection was rare in individuals above 40 years of age. In contrast, confirmed hepatitis E afflicted mainly old individuals and the infection was rare among individuals under 20 years of age. The age distribution in 54 presumptive co-infection cases positive for IgM HAV and IgM HEV [G], but negative for other HEV, was essentially

the same as that in those with confirmed hepatitis A ($\chi^2=4.95$, $P=0.667$), but it was different from the age distribution in cases with confirmed hepatitis E ($\chi^2=28.8$, $P<0.001$), suggesting that these co-infection cases were affected with hepatitis A. On the other hand, the age distribution in the other 18 co-infection cases positive for IgM HAV and IgM HEV [E] was essentially the same as that in those with confirmed hepatitis E ($\chi^2=38.62$, $P<0.001$), but different from age distribution of hepatitis A ($\chi^2=0.85$, $P=0.99$), suggesting that these cases were affected with hepatitis E.

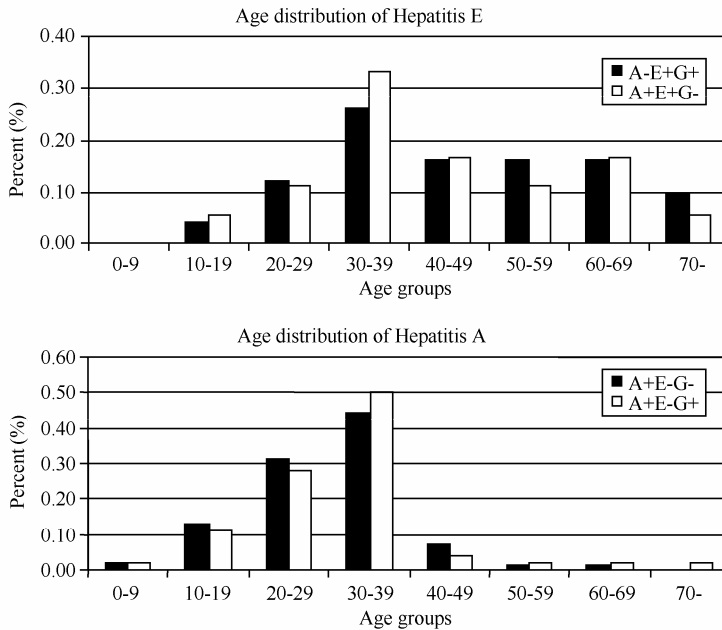


FIG. 1. Age distribution in patients with enteric viral hepatitis. The cases included 187 with confirmed hepatitis A, 50 with confirmed hepatitis E and 72 with co-infection. Solid bar depicts the cases with confirmed hepatitis E or hepatitis A, and open bar depicts the cases with co-infection.

Differential diagnosis of 315 enteric hepatitis cases is summarized in Table 2. These cases included 187 with a confirmed diagnosis of hepatitis A based on their positiveness for HAV IgM and the diagnosis was supported by exclusion of hepatitis E. Another 54 cases were considered to have a presumptive diagnosis of hepatitis A based on their positiveness for HAV IgM and their age distribution. The 50 cases were considered having a confirmed diagnosis of hepatitis E based on their positiveness for anti-HEV IgM. The diagnosis was supported by exclusion of hepatitis A. Six cases were considered having a presumptive diagnosis of hepatitis E based on their positiveness for anti-HEV IgM. The diagnosis was supported by exclusion of hepatitis A. The other 18

cases were positive for HEV IgM. Although they were also positive by HAV IgM, they were nevertheless considered as having a presumptive diagnosis of hepatitis E, because their age distribution was the same as that in those with hepatitis E, but different from that in those with hepatitis A. HEV IgM [E] was positive in all the confirmed hepatitis E cases, 22 presumptive hepatitis E cases and anti-HEV IgM was negative in all 241 confirmed or presumptive hepatitis A cases. HEV IgM [G] was found to be positive in all 50 confirmed hepatitis E cases, 2 presumptive hepatitis E cases and 54 presumptive hepatitis A cases. These results indicate that the sensitivity of HEV IgM [E] and HEV IgM [G] is 97% and 70%, respectively.

TABLE 2

Performance of IgM Anti-HEV ELISA

Diagnosis	Cases (n)		
	All	HEV IgM [E] Positive	HEV IgM [G] Positive
Hepatitis A	187	0	0
Presumptive Hepatitis A	54	0	54
Total Hepatitis A	241	0	54
Hepatitis E	50	50	50
Presumptive Hepatitis E	24	22	2
Total hepatitis E	74	72	52

Note. In hepatitis E, the sensitivity of HEV IgM [E] and HEV IgM [G] was 97.3% (72/74) and 70.3% (52/74) respectively. In hepatitis A, the specificity of HEV IgM [E] and HEV IgM [G] was 100% (1-0/241) and 77.6% (1-54/241) respectively.

DISCUSSION

HAV and HEV are principal causes of acute hepatitis in Asia and Africa where both viruses are prevalent. The disease is mainly attributed to immunopathology brought about by the infection rather than the infection per se. In primates experimentally challenged by the viruses, viremia usually precedes, while antibody response usually coincides, onset of the diseases. In humans, onset of disease is usually insidious and patients are often presented at relatively advanced disease stage when viral activity has subsided to various extent and antibody has reached is or near peak level. Consequently, the conventional approach to diagnosis based on detection of virus and/or rising antibody level in paired sera samples are not appropriate for diagnosis of acute viral hepatitis. Instead, IgM antibodies against the respective viruses are considered to be more appropriate for diagnosis of viral hepatitis than the conventional methods and hence were used as the primary acute markers of infection in the present study, because levels of these antibodies rise immediately before disease onset and persist throughout acute phase and convalescence.

The study was conducted in Southern China, where both viruses co-circulates, and the HEV population is dominated by zoonotic genotype 4 virus. Whereas the main reservoir of HAV is humans and it afflicts mainly children and young adults, HEV afflicts mainly adults and elderly. Co-infection by both viruses is less certain and is considered unlikely because the reservoirs of the respective viruses are different. According their epidemiology, these presumptive co-infections can be distinguished as hepatitis A or hepatitis E. The reason why a higher specificity can be achieved by HEV IgM [E] is that the assay makes use of IgM capture test format, and the higher sensitivity achieved by this assay is attributed largely to its choice of HEV antigen. Our results indicate that this assay is useful in facilitating differential diagnosis of sporadic cases of enteric hepatitis.

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