SUSCEPTIBILITY TO HEPATITIS A VIRUS INFECTION AMONG CHRONIC LIVER DISEASE PATIENTS AND HEALTHY BLOOD DONORS IN THAILAND

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Abstract. Due to improvements in socio-economic and sanitation conditions, Thailand has undergone a change from hyperendemicity to intermediate endemicity for hepatitis A virus infection, leaving a large part of the adult population without immunity. At the same time, the country is still highly endemic for hepatitis B and especially in the northeast, hepatitis C virus infection both of which when acquired during infancy or early childhood exhibit a strong tendency to turn towards chronic liver disease, although in particular with hepatitis B virus the asymptomatic carrier state is also rather common. As no cross-immunity exists between any of these viruses, double or triple infections do occur, a situation where previously acquired immunity to HAV becomes crucial as double infections have been shown to take a more severe or even fatal course. In the present study, we investigated 820 HBV- and/or HCV-related chronic liver disease (CLD) patients and 195 blood donors, both groups divided by 10-year age intervals, for the prevalence of anti-HAV. The results showed the same age dependence of immunity for all groups tested as can be expected for an area of intermediate endemicity, in that approximately 50 % of those between 21 and 30 years of age had acquired anti-HAV. These findings indicate the immune response to HAV infection not to be altered by chronic infection with either HBV or HCV. Hence, vaccination against HAV should be considered, particularly in anti-HAV-negative patients with CLD.

INTRODUCTION

To this day, hepatitis A virus (HAV) infection represents a global public health burden of major dimensions, particularly in developing countries on the verge of industrialization where, due to advanced hygiene and sanitation, the pattern of infection has undergone a gradual alteration from hyper- to hypoendemic. Contrasting the situation in undeveloped areas where infection usually occurs during infancy or early childhood, following almost without exception a subclinical course and subsequently leaving the individual with permanent immunity, improved socio-economic conditions in developing countries have led to an ever increasing number of adolescents and young adults susceptible to the virus. As hepatitis A can culminate in acute forms of liver disease including rare cases of fulminant hepatitis and as the infection potentially turns more severe with increasing age of the host, the age at which natural immunity is acquired will directly affect the ratio of clinical versus subclinical disease patterns (Lednars *et al*, 1985; Hadler, 1991).

Moreover, because of the relatively high incidence of hepatitis viruses A, B and C (HAV, HBV, HCV) sequential or simultaneous infections with any combination of these viruses are common, especially since none induces cross-immunity. Still, reliable data regarding *in vivo* interference of these viruses have been quite scarce (Drucker *et al*, 1979; Hindman *et al*, 1977; Krugman *et al*, 1974) and hence, it has widely remained a subject of speculation whether double or multiple viral hepatitis infection does actually clinically differ from infection with either virus on its own.

Isolated HAV infection, unless occurring at a rather advanced age, is rarely fatal (Hadler, 1991)

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and similarly, it seldom proceeds towards fulminant hepatic failure, unless the patient in question already has been suffering from underlying chronic liver disease due to HBV and/or HCV infection or otherwise, is immunocompromised (Lemon, 1985). On the other hand, superinfection with hepatitis A virus occurring in patients with chronic liver disease of viral etiology may take a much more severe course associated with more pronounced liver enzyme abnormalities and ensuing higher mortality rate due to fulminant hepatic failure (Wang *et al*, 1986; Yao, 1991). For example, during the acute hepatitis A epidemic in Shanghai, 1988, 31.9 % of the deaths reported were caused by hepatitis A superimposed on chronic hepatitis B (Yao, 1991). Similarly, 28 % of the deaths occurring among hepatitis A patients in the United States between 1983 and 1988 were a consequence of underlying chronic liver disease other than alcoholic cirrhosis (Hadler, 1991). Both examples suggest either synergism of both viruses or else, some as yet unknown biochemical mechanism triggered by co-infection, leading to a more severe disease course and hence, implying a heightened risk.

The disease process of hepatitis A in patients with chronic hepatitis B has been discussed in various studies (Keeffe, 1995), and recently been reported to take an even more severe course in patients with chronic hepatitis C (Vento *et al*, 1998). Yet, it is presently unknown whether the HBV and/or HCV carrier state and underlying chronic liver disease influence the course of hepatitis A and similarly, documented attacks of acute hepatitis A in chronic HBsAg carriers with biopsy-ascertained liver disease and the outcome of those double infections are rare. Although assessing the impact of CLD on the severity of HAV superinfection is exceedingly difficult, based on an estimated 2 % prevalence of CLD in the USA, the case fatality rate with these patients can be tentatively calculated at 4.6 %, exceeding that of patients without liver disease by more than 20 fold.

As in Thailand the probability for any individual to be infected simultaneously or in succession by any combination of hepatitis viruses is quite considerable and also, as natural immunity to HAV has been steadily declining over the past two decades (Poovorawan *et al*, 1993, 1997), we conducted the present study in order to screen asymptomatic HBV carriers, HBV- and HCV-related CLD patients belonging to different age groups for antibodies to hepatitis A virus. In order to differentiate between the expected age dependence of anti-HAV and any potential impact of the underlying CLD, 195 healthy blood donors served as controls.

MATERIALS AND METHODS

Study population and patients

We investigated 820 adults (557 males, 263 females), their mean age ranging from 32 ± 11.9 to 66 ± 9.6 years, with regards to anti-HAV prevalence. Of these, 507 were asymptomatic HBV carriers, 196 had been diagnosed with HBV-related CLD, and 117 with HCV-related CLD, respectively. Those 313 patients diagnosed with HBV-or HCV-related chronic liver disease confirmed by clinical and/or biochemical parameters, as well as by liver biopsy, had been attending Ramathibodi and Phaya Thai Hospital. For the purpose of the study, the patients were divided into 4 groups:

1. Asymptomatic HBV carriers: Serum samples had been collected from altogether 507 asymptomatic HBV carriers residing in Bangkok proper. Of these, 339 (66.9 %) were males, and 168 (33.1 %) females, their age ranging from 17 to 61 years (mean age 32 ± 11.9 years).

2. HBV-related chronic liver disease: This group comprised a total of 196 patients positive for HBsAg and exhibiting abnormal LFT with a male predominance of 77.5 % (152/196) over 22.4 % (44/196) females, their mean age ranging between 50.8 ± 13.8 and 53.8 ± 11 years. The patients were classified according to liver histology as chronic hepatitis (115 cases), liver cirrhosis (31 cases), and hepatocellular carcinoma (HCC) (50 cases). The characteristics of the patients are summarized in Table 1.

3. HCV-related chronic liver disease: There were a total of 117 patients positive for anti-HCV and HCV RNA and showing elevated LFT levels, 56.4 % (66/117) of who were males, and 43.6 % (51/117) were females, with a mean age between 50.8 ± 13.8 and 66.0 ± 9.6 years. Of these, 65.8 % (77/117) were infected by HCV genotype II/1b, 28.2 % (33/117) by genotype III/2a, and 5.98 % (7/117) showed mixed infection by genotype II and III, respectively. Based on liver histology, 82 had been diagnosed with chronic hepatitis, 25 with liver cirrhosis, and 10 with HCC. The characteristics of the patients are summarized in Table 1.

4. Control group: A total of 195 healthy blood donors all of who were negative for HBsAg and anti-HCV, 147 males and 48 females, with a mean age of 32.6 ± 10.4 years served as controls. All

subjects tested were divided into age groups at 10-year intervals. Blood samples were collected for complete blood count, blood biochemistry, viral serology and liver function test.

Laboratory tests

Serological tests : HBsAg was measured using a viral serological marker supplied with commercially available enzyme radioimmunoassay kits (Abbott Laboratories, North Chicago, Ill, USA). Anti-HCV in serum was detected by the third-generation enzyme-linked immunosorbent assay (ELISA) kit (Abbott Laboratories). Screening for anti-HAV IgG was performed by a commercially available ELISA kit (HAVAB, Abbott Laboratories, North Chicago, Ill, USA). Cut-off points were determined according to the manufacturer's specifications.

Histopathological diagnosis

Liver biopsy: All chronic HBV and HCV patients giving their oral informed consent underwent liver biopsy between January 1990 and 1997. Biopsy findings were scored according to the Histologic Activity Index proposed by Knodell for classification (Knodell *et al,* 1981).

RESULTS

Prevalence of total anti-HAV in asymptomatic HBV carriers

Among the 507 asymptomatic HBsAg carriers tested 41.56 % (32 out of 77) of those aged below 20 years displayed immunity to hepatitis A virus infection as demonstrated by the presence of anti-HAV IgG. Antibody prevalence steadily increased in direct proportion to the respective age group in that among those between 21 and 30 years of age it amounted to 56.15 % (105 out of 187), in the age group between 31 and 40 years to 80 % (88 out of 110), between 41 and 50 years to 91.4 % (85 out of 93), between 51 and 60 years to 94.12 % (32 out of 34), until among those above the age of 60 years it had attained 100 % (6 out of 6).

Prevalence of total anti-HAV in HBV-related CLD

In the group of HBV-related chronic hepatitis patients, total anti-HAV IgG was shown at its minimum of 16.67 % within the age group of below 20 years (1 out of 6). Thereafter, immunity to hepatitis A virus rose to 42.10% within the age group between 21 and 30 years (8 out of 19), 87.87 % among those between 31 and 40 years (29 out of 33), and 90.62 % in patients aged between 41 and 50 years (29 out of 32), respectively. The prevalence of anti-HAV underwent yet another increase among patients between 51 and 60 years (12 out of 13) and finally attained its maximum of 100 % in those above 60 years (5 out of 5).

Prevalence of total anti-HAV in HCV-related CLD

Among the patients with HCV-related CLD, total anti-HAV IgG was found to be at a minimum of 50 % in the age group of between 21 and 30 years (1 out of 2). With patients aged between 31

Table 1
The prevalence of total anti-HAV in asymptomatic HBV carriers, patients with HBV-/HCV-related chronic liver disease and blood donors.

Group of patients	No. of patients	Age (years)	Sex Male : female	Patients with positive anti-HAV	
				n	%
HBV carriers	507	32 ± 11.9	339 : 168	348	68.6
HBV-related CLD	196	50.8 ± 13.8	152 : 44	162	82.6
Chronic hepatitis	115	40.2 ± 12.2	89 : 26	87	75.6
Cirrhosis	31	54.0 ± 9.8	21 : 10	27	87.1
HCC	50	53.8 ± 11.0	42 : 8	48	96.0
HCV-related CLD	117	50.8 ± 13.8	66 : 51	106	90.6
Chronic hepatitis	82	45.9 ± 11.1	45 : 37	73	89.0
Cirrhosis	25	60.6 ± 13.9	15 : 10	23	92.0
HCC	10	66.0 ± 9.6	6 : 4	10	100
Blood donors	195	32.6 ± 10.43	138 : 57	126	64.6

and 40 years it reached 82.14 % (23 out of 28), and with those between 41 and 50 years 89.66 % (26 out of 29), respectively. Total antibody to hepatitis A virus was demonstrated to peak with 100 % prevalence in both the age groups between 51 and 60 years (13 out of 13) and above 60 years (10 out of 10), respectively.

Prevalence of total anti-HAV in healthy blood donors

In the group of healthy blood donors, anti-HAV IgG was detected at its minimum of 32 % within the age group below 20 years (8 out of 25), rising to 44.9 % among those between 21 and 30 years (31 out of 69), increasing again to 76.4 % among those aged 31 to 40 years (42 out of 55), approaching its maximum with 97 % within the age group of 41 to 50 years (32 out of 33), and levelling out at 100 % among those between 51 and 60 years (12 out of 12) and above (1 of 1). The prevalences of anti-HAV among the different groups studied were comparable and increasing with age. The details are shown in Fig 1.

DISCUSSION

Due to improvements in socio-economic conditions and hygiene Thailand, formerly hyperendemic for hepatitis A (HAV) virus infection, has experienced a change towards intermediate endemicity. Hence, whereas in the past infections used to occur during infancy or early childhood when they had almost without exception an asymptomatic course and left the host with life-long immunity, nowadays susceptible hosts are found within the higher age groups, namely, adolescents and adults who, when infected develop hepatobiliary symptoms of varying severity. Yet, hepatitis A on its own is rarely fatal and has not been reported to cause fulminant hepatic failure.

Since Thailand is highly endemic for hepatitis B (HBV) and, particularly in the northeastern part of the country hepatitis C virus (HCV) infection, pre-existing immunity to hepatitis A virus becomes crucial for the numerous individuals chronically infected with HBV and/or HCV and superinfected with HAV as this coinfection can trigger more severe symptomatology and a possibly fatal outcome. Although the data reported to date are somewhat conflicting, mostly depending on the geographical area where the respective studies have been performed (Papachristou and Dumas, 1991; Zachoval *et al*, 1983), two important indicators as to potential severity of HAV coinfection can be discerned. First, clinical manifestations and course of hepatitis among asymptomatic chronic HBV carriers are not different from that in HBsAg negative patients, whereas superinfection of HAV and chronic active hepatitis B mostly results in the exacerbation of

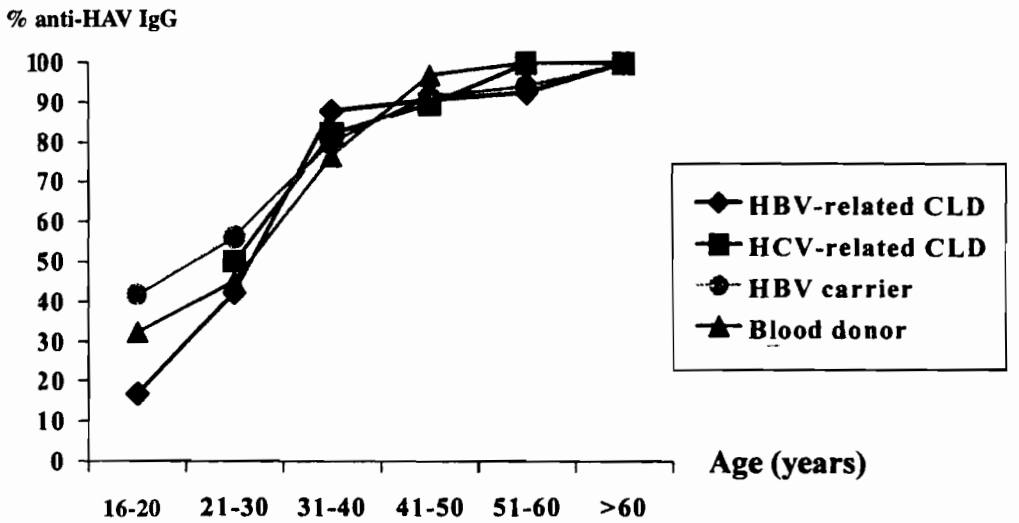

Fig 1–Percentage of anti-HAV in asymptomatic HBV carriers, HBV- and HCV-related chronic liver disease patients and blood donors among different age groups.

liver disease with subsequent ascites and liver failure (Yao, 1991). Second, patients with chronic hepatitis C carry a substantial risk of fulminant hepatitis and death associated with HAV superinfection (Vento *et al,* 1998).

The present study was aimed at elucidating if the immune response to HAV infection is in any way affected by chronic infections with either HBV or HCV or otherwise, if it exhibits an age-dependent prevalence indicative of an area of intermediate endemicity. Our results clearly show immunity to HAV infection increasing with age, irrespective of the group of individuals tested. In other words, anti-HAV was found to gradually increase within the age group between 21 and 30 years comprising asymptomatic HBV carriers, HBV- as well as HCV-related CLD patients and healthy blood donors alike, reaching an approximately 80 % protective level among those between the age of 31 and 40 years. Consequently, HBV-/HCV-related CLD patients below the age of 20, a scenario not uncommon in Thailand, are at increased risk to develop fulminant hepatic failure due to superinfection with HAV. Hence, we would recommend those individuals diagnosed with chronic liver disease due to chronic HBV/HCV infection and negative for anti-HAV be vaccinated against hepatitis A virus infection.

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REFERENCES

Drucker J, Tabor E, Gerety RJ, *et al.* Simultaneous acute infections with hepatitis A and hepatitis B viruses in a chimpanzee. *J Infect Dis* 1979; 139: 338-42.

Hadler SC. Global impact of hepatitis A virus infection: changing patterns. In: Hollinger FB, Lemon SM, Margolis HS, eds. Viral hepatitis and liver disease. Baltimore: Williams and Wilkins, 1991; pp 14-20.

Hindman SH, Maynard JE, Bradley DW, *et al.* Simultaneous infection with type A and B hepatitis viruses. *Am J Epidemiol* 1977; 105: 135-9.

Keeffe EB. Is hepatitis A more severe in patients with chronic hepatitis B and other chronic liver diseases? *Am J Gastroenterol* 1995; 90: 201-5.

Knodell RG, Ishak KG, Black WC, *et al.* Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; 1: 431-5.

Krugman S, Hoofnagle J, Gerety RJ, Kaplan PM, Gerin JL. Viral hepatitis, type B DNA polymerase activity and antibody to hepatitis B core antigen. *N Engl J Med* 1974; 290: 1331-5.

Lednar WM, Lemon SM, Kirkpatrick JW, Redfield RR, Fields ML, Kelley PW. Frequency of illness associated with epidemic hepatitis A virus infection in adults. *Am J Epidemiol* 1985; 122: 226-33.

Lemon SM. Type A viral hepatitis. New developments in an old disease. *N Engl J Med* 1985; 313: 1059-67.

Papachristou AA, Dumas AS, Katsouyannopoulos VC. Dissociation of alanine aminotransferase values in acute hepatitis A patients with and without past experience to the hepatitis B virus. *Epidemiol Infect* 1991; 106: 397-402.

Poovorawan Y, Theamboonlers A, Chumdermpadetsuk S. Changing seroepidemiology of hepatitis A virus infection in Thailand. *Southeast Asian J Trop Med Public Health* 1993; 24: 250-4.

Poovorawan Y, Vimolkej T, Chongsrisawat V, Theamboonlers A, Chumdermpadetsuk S. The declining pattern of seroepidemiology of hepatitis A virus infection among adolescents in Bangkok, Thailand. *Southeast Asian J Trop Med Public Health* 1997; 28: 154-7.

Vento S, Garofano T, Renzini C, *et al.* Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *N Engl J Med* 1998; 338: 286-90.

Wang J-Y, Lee S-D, Tsai Y-T, *et al.* Fulminant hepatitis A in chronic HBV carrier. *Dig Dis Sci* 1986; 31: 109-11.

Yao G. Clinical spectrum and natural history of viral hepatitis A in a 1988 Shanghai epidemic. In: Hollinger FB, Lemon SM, Margolis D, eds. Viral hepatitis and liver disease. Baltimore: Williams and Wilkins, 1991; 76-8.

Zachoval R, Roggendorf M, Deinhardt F. Hepatitis A infection in chronic carriers of hepatitis B virus. *Hepatology* 1983; 3: 528-31.