THE PREVALENCE OF VIRAL HEPATITIS AMONG THE HMONG PEOPLE OF NORTHERN THAILAND

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Abstract. Sera from 269 Hmong people (102 males and 167 females, with mean age 35.4 years, range 16-63 years) were examined in order to determine the seroprevalence of hepatitis virus infection. The seroprevalence rates for HAV (hepatitis A virus), HBV (hepatitis B virus), HCV (hepatitis C virus), HDV (hepatitis D virus), HEV (hepatitis E virus), HGV (hepatitis G virus) and TTV (TT virus) infection were 87.8% (n=140), 76.0% (n=150), 2.0% (n=150), 0.7% (n=150), 6.5% (n=139), 5.3% (n=94) and 25.6% (n=121) respectively. The rate for carriers of HBV (HBsAg) was 13.8 % (20.5% in males and 9.6% females) with a peak prevalence in the 21-40 year age group. A high rate of HAV seropositivity was found among the younger subjects. The rate of HEV seroprevalence was low. The prevalence of TTV-DNA was high with no difference between the sexes. HGV-RNA prevalence was low and seen primarily in males. This study indicates that the Hmong people are endemically infected with HAV and HBV infection and should be considered for targeted vaccination. The role of TTV and HGV in producing illness and hepatic disease has yet to be determined in this population.

INTRODUCTION

During the last decade, more information about new hepatitis viruses, including hepatitis G virus (HGV), TT virus (TTV) and SEN virus (SENV) - all associated with transfusions - has emerged (Summerfield, 2000; Bowden, 2001). In addition to transfusion-acquired infection, it is probable that these viruses are spread by non-parenteral modes of transmission. Hepatitis A virus (HAV) and hepatitis E virus (HEV) are transmitted by the fecal-oral route and cause self-limiting infections. Hepatitis B virus is (HBV) transmitted by parenteral, vertical and sexual routes, whereas hepatitis C virus (HCV) is usually transmitted by transfusion. Both HBV and HCV can cause chronic infections. The transmission of HDV is dependent upon HBV and HBV envelop proteins. TTV

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can be transmitted by the fecal-oral route as well as parenterally.

The predominance of HAV infection in developing countries, including Thailand, has declined in association with improvements in standards of living and socioeconomic conditions (Poovorawan *et al*, 1997; Kunasol *et al,* 1998). In the early 1980s the prevalence of anti-HAV was 85-95% at age 10-15 years in Thailand. By the late 1990s such a high rate of prevalence was found only among those over 30 years of age after sustained economic growth and widespread improvements in sanitation (Barzaga, 2000).

The prevalence of HBV infection increases with age, with a carrier rate of 5-10% in Thai adults (Ervuchavarakul *et al*, 1989; Merican *et al*, 2000). However, the overall prevalence rate of hepatitis B surface antigen (HBsAg) was reduced to 0.55% after a massive campaign of vaccination under the Expanded Program of Immunization (EPI) (Hub-uppakarn *et al*, 1998).

A high seroprevalence of HDV and HCV infection is found in injecting drug users (IDUs) with rates of 60% (Louisirirotchanakul *et al*, 1988) and 90% (Wasi *et al*, 1990; Luksamijarulkul and Pucktaweesak, 1996) respectively. The prevalence of (HGV/GB virus C) infection among IDUs has been reported as being 22.5% (Vimolket *et al,* 1998). In addition, HEV infection occurs in Thai children and adolescents at <10% (Poovorawan *et al*, 1996). A high prevalence of TTV infection (36%) has been reported in Thai blood donors (Tanaka *et al*, 1998).

All of this epidemiological information was obtained by studies in the central part of Thailand. No information has been published regarding viral hepatitis among minority peoples such as the Hmong, a distinct ethno-linguistic group living in the highlands of northern Thailand. Over 9 million Hmong live in China and northern Southeast Asia; some 100,000 ethnic Hmong reside in Thailand. This study aimed to determine the distribution of viral hepatitis caused by HAV, HBV, HCV, HDV, HEV, HGV and TTV in the Hmong population of northern Thailand.

MATERIALS AND METHODS

Subjects

The subjects were ethnic Hmong living in two highland farming communities about 30 km from Chiang Mai. Samples were collected during a comprehensive health survey of rural and urban ethnic Hmong in northern Thailand that was conducted by the Research Institute for Health Sciences, Chiang Mai University, and the University of California, San Francisco, in 2000.

Randomly selected sera from a set of 150 subjects, all of whom had given their informed consent, were used for the study of the prevalence of viral hepatitis at Siriraj Hospital, Bangkok, Thailand, except HEV which were studied at Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. The subjects comprised 63 males and 87 females with mean of age 35 (range 16-63) years. Sera were separated and kept at -70ºC until use. Data from other aspects of this study were published elsewhere (Louisirirotchanakul *et al*, 2000; Kunstadter *et al*, 2001).

For the study of HBV carriage, further serum samples were examined. These samples were drawn from people of the villages of KCK (Khun Chang Khian) (n=134) and MSM (Mae Sa Mai) (n=135). There were 55 males and 79 females (mean age 35.4; range 16-63 years) in the first village and 47 males and 88 females (mean age 33.9; range 15-60 years) in the second village.

Methods

Anti-HAV, HBsAg and anti-HBs were measured by enzyme-linked immunosorbent assay (EIA, Organon Teknika, Netherlands); anti-HCV and anti-HDV were detected by EIA (Abbott, Europe; UBI, USA respectively). The EIA method for anti-HAV, anti-HCV and anti-HEV (Innis *et al*, 2002) was indirect; HBsAg, anti-HBs and anti-HDV were assessed by a double sandwich technique.

Serum HGV RNA was determined by nested RT-PCR (reverse transcriptase polymerase chain reaction) as previously described (Linnen *et al*, 1996). Briefly, RNA was extracted from 100 µl serum using TRIzol LS (GibcoBRL, #10296-010) and chloroform, then, resuspended in 25 µl deionized water. The reverse transcription was performed in two steps: denaturing and RT. Firstly, 2.5 µl of the RNA suspension was denatured at 95ºC for 5 minutes with 1 µl of 10 pmol/µl HGV-7116 primer (5′-GAG-CCA-CGT-TGA-AGA-CAC-TT-3′) and 1.5 µl of DEPC-treated water; secondly, the denatured RNA was transcribed into cDNA at 42ºC for 90 minutes by mixing with the reverse transcription reagents, which consisted of 4 µl of 2.5 mM dNTPs, 0.5 µl of 40 unit RNAse inhibitor (Promega, USA), 2.5 µl of 5x RT buffer (Promega supplied), 0.15 µl of 10 unit AMV-RT (Promega, USA) and 0.35 µl RNase-free water.

The HGV cDNA was amplified by nested PCR. For the first round of PCR, 12.5 µl

cDNA was mixed with PCR mixture, which consisted of 1.5 µl of 10 pmol/µl HGV-7116 primer (5′-GAG-CCA-CGT-TGA-AGA-CAC-TT-3′), 2.5 µl of 10 pmol/µl HGV-6842 primer (5′-GAA-TGC-TGC-GAG-GAT-TCT-TG-3′), 3.75 µl of 10x *Ex Taq^TM* buffer (including MgCl$_2$, TaKaRa, Japan), 0.25 µl of 5U/µl TaKaRa *Ex Taq^TM* polymerase (TaKaRa, Japan), and DEPC-treated water to give the final volume of 50 µl. For the second round of PCR, 2.5 µl (1:50) of the first amplified product was mixed with PCR mixture, which consisted of 2.5 µl of 10 pmol/µl HGV-6904 primer (5′-CTC-TTT-GTG-GTA-GTA-GCC-GAG-AGA-T-3′), 2.5 µl of 10 pmol/µl HGV-7059 primer (5′-CGA-ATG-AGT-CAG-AGG-ACG-GGG-TAT-3′), 5 µl of 10x *Ex Taq^TM* buffer (including MgCl$_2$, TaKaRa, Japan), 0.25 µl of 5U/µl TaKaRa *Ex Taq^TM* polymerase (TaKaRa, Japan), 4.0 µl of 2.5 mM dNTPs and DEPC-treated water to give the final volume of 50 µl. PCR was carried out by a Gene Amp PCR System 9700 Thermal Cycler with denaturation at 95ºC for 5 minutes. For the first round, the condition step was at 94ºC for 1 minute, 55ºC for 2 minutes, 72ºC for 1 minute (5 cycles); 94ºC for 1 minute, 55ºC for 1 minute, 72ºC for 1 minute (24 cycles); and followed at 94ºC for 1 minute, 55ºC for 1 minute, 72ºC for 6 minutes (1 cycle). For the second round, the condition step was at 94ºC for 1.5 minutes, 55ºC for 1 minute, 72ºC for 1 minute (1 cycle); 94ºC for 1 minute, 55ºC for 1 minute, 72ºC for 1 minute (28 cycles); and 94ºC for 1 minute, 55ºC for 1 minute, 72ºC for 5 minutes (1 cycle) and soaking at 4ºC. The final product of 156 bp was separated by 1.5% agarose gel electrophoresis (ethidium bromide stained). The gel was visualized by UV-translumination.

Serum TTV DNA was detected by nested PCR as previously described (Naoumov *et al*, 1998). Briefly, DNA was extracted from 100 µl serum using phenol-chloroform and proteinase K. The precipitated sample was resuspended in 25 µl deionized water and amplified by nested PCR. For the first round of PCR, 10 µl DNA extraction was mixed with PCR mixture, which consisted of 1 µl of 10 pmol/µl primer A (sense 5′-ACA GAC AGA GGA GAA GGC AAC ATG-3′) and 1 µl of 10 pmol/µl primer B (anti-sense 5′CTG GCA TTT TAC CAT TTC CAA AGT T-3′), 4.0 µl of 2.5mM dNTPs (Promega, USA), 5.0 µl of 10xPCR buffer (Promega, USA), 4.0 µl of 25 mM MgCl$_2$, 0.5 µl of 5 U/µl *Taq* DNA polymerase (Promega, USA) and deionized water to give the final volume 50 µl. For the second round of PCR, 1 µl of amplified product was mixed with the same PCR mixture including the primer B but using the primer C (5′ GGC AAC ATG TTA TGG ATA GAC TGG 3′) instead of primer A. The PCR was performed by a Gene Amp PCR System 9700 Thermal Cycler with the conditions of 35 cycles in the first round and 25 cycles in the second round at 94ºC for 30 seconds, 58ºC for 30 seconds, and 72ºC for 45 seconds, and then extension at 72ºC for 7 minutes. The final amplified product of 272 bp was observed by UV-translumination.

Statistical analysis

Fisher's exact test (p<0.05) was used to assess the statistical significance of the differences in the prevalence of hepatitis between the sexes.

RESULTS

The prevalence of viral hepatitis was found to be 87.9% HAV (123 of 140), 76.0% HBV (114 of 150 by either HBsAg or anti-HBs), 25.6% TTV (31 of 121), 6.5% HEV (9 of 139), 3.2% HGV (3 of 94), 2% HCV (3 of 150) and 0.7% HDV (1 of 150) as shown in Fig 1.

There was a significant difference in the prevalence of anti-HAV between female and male subjects (93.9% *vs* 79.3%, p= 0.016, odds ratio=4) but no significant between sex differences were observed for the other viral markers (p>0.05) (Table 1).

The second highest prevalence of viral hepatitis infection was HBV, indicated by HBsAg (14.6%; 22 of 150) and anti-HBs (61.3%; 92 of 150). HBV prevalence was associated with age (Table 1). Interestingly, greater HBsAg positivity (20.6%; 13 of 63)

Table 1
Distribution of viral hepatitis markers among Hmong population by age and gender[a].

Age (year)	%Anti-HAV[b] n=140		%HBs Ag n=150		%Anti-HBs n=150		%Anti-HCV n=150		%Anti-HDV[c] n=23		%Anti-HEV n=139		HGV-RNA n=94		TTV-DNA n=121	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
11-20	100.0 (8/8)	100.0 (6/6)	12.5 (1/8)	16.7 (1/6)	50.0 (4/8)	50.0 (3/6)	0 (0/8)	0 (0/6)	0 (0/1)	0 (0/1)	12.5 (1/8)	0 (0/6)	0 (0/6)	0 (0/3)	0 (0/6)	25.0 (1/4)
21-30	86.9 (20/23)	64.7 (11/17)	16.7 (4/24)	15.8 (3/19)	79.2 (19/24)	52.6 (10/19)	0 (0/24)	5.3 (1/19)	0 (0/4)	0 (0/3)	0 (0/23)	0 (0/16)	0 (0/13)	13.0 (1/8)	18.0 (3/17)	23.5 (4/17)
31-40	89.5 (17/19)	77.8 (14/18)	8.7 (2/23)	35.0 (7/20)	78.0 (18/23)	45.0 (9/20)	0 (0/23)	5.3 (1/20)	0 (0/2)	14.0 (1/7)	0 (0/19)	16.7 (3/18)	6.3 (1/16)	7.0 (1/15)	19.0 (4/21)	35.0 (6/17)
41-50	100.0 (23/23)	88.9 (8/9)	4.3 (1/23)	20.0 (2/10)	43.5 (10/23)	70.0 (7/10)	4.3 (1/23)	0 (0/10)	0 (0/1)	0 (0/2)	13.0 (3/23)	22.2 (2/9)	7.1 (1/14)	0 (0/6)	33.0 (6/18)	28.6 (2/9)
51-60	100.0 (9/9)	100.0 (8/8)	11.1 (1/9)	0 (0/8)	55.6 (5/9)	87.5 (7/8)	0 (0/9)	0 (0/8)	0 (0/2)	0 (0/0)	0 (0/9)	0 (0/8)	0 (0/6)	14.3 (1/7)	28.6 (2/7)	42.9 (3/7)
Total	93.9 (77/82)	79.3 (46/58)	10.3 (9/87)	20.6 (13/63)	64.4 (56/87)	57.1 (36/63)	1.1 (1/87)	3.2 (2/63)	0 (0/10)	7.7 (1/13)	4.9 (4/82)	8.8 (5/57)	3.6 (2/55)	7.7 (3/39)	21.7 (15/69)	30.8 (16/52)

[a]The data is expressed as percentage of detection (No. pos/ No. test)
[b]A significant difference of the prevalence of anti-HAV between the sexes was found (p<0.05; Fisher's exact test).
[c]Anti-HDV in the presence of HBV antigenemia; HDV markers were considered to be at 0% in female and 1.59% in male groups.

Table 2
HBV carriage in 2 Hmong villages.

Age (year)	%HBsAg (No. detected /No. tested)			
	KCK village		MSM village	
	Male	Female	Male	Female
11-20	33.3 (1/3)	11.1 (1/9)	40.0 (2/5)	0 (0/12)
21-30	15.4 (2/13)	25.0 (6/24)	21.4 (3/14)	12.0 (3/25)
31-40	31.6 (6/19)	4.5 (1/22)	13.3 (2/15)	11.1 (3/27)
41-50	20.0 (2/10)	5.8 (1/17)	14.3 (1/7)	0 (0/15)
51-60	12.5 (1/8)	0 (0/7)	20.0 (1/5)	11.1 (1/9)
> 61	0 (0/2)		0 (0/1)	
Total	21.8 (12/55)	11.3 (9/79)	19.1 (9/47)	7.9 (7/88)
Overall	15.6 (21/134)		11.8 (16/135)	

was observed in males, with the peak in the 31-40 year age group (35%); lower antigenemia (10.3%; 9 of 87) was found in the females, with the highest rate in the 21-30 year age group (16.7%). In order to confirm the rate of carriage of HBV among the ethnic Hmong population, samples from the two villages were analyzed (Table 2). As expected, the HBsAg positivity was 15.7% and 11.9% in KCK and MSM villages respectively. Males were more likely to be carriers than females (22% *vs* 11% in KCK; 19% *vs* 8% in MSM). The overall carriage of HBV in this Hmong population was 13.8% (37 of 269), with 20.5% (21 of 102) in males and 9.6% (16 of 167) in females.

Because HBsAg is required for HDV replication, anti-HDV was performed only for subjects with HBs antigenemia. Only 1 of the 23 (7.7%) males and none of the 10 females with HBV viremia had simultaneous HDV infection (Table 1). Therefore, the overall rate of HDV infection should be considered to be 0.7% (1 of 150) (Fig 1).

The seroprevalence of anti-HCV was 2.0% (3 of 150). Chronic HCV infection could not be identified in this limited number of samples.

The overall seropositivity of anti-HEV was low in both females (4.9%; 4 of 82) and males (8.8%; 5 of 57) (Table 1).
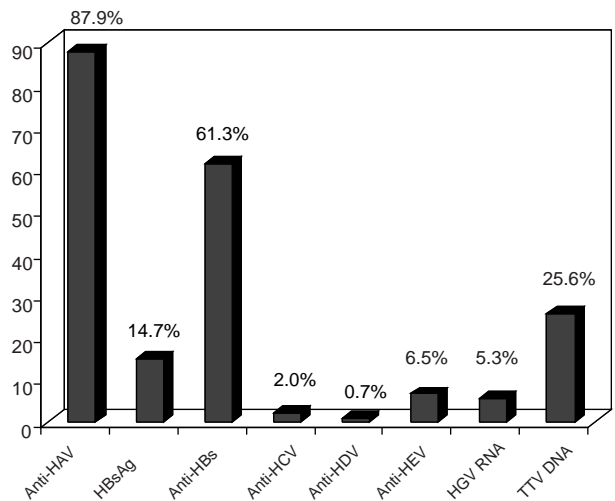
There was a high prevalence of TTV DNA

Fig 1–Frequency of viral hepatitis markers in the Hmong population.

viremia (25.6%; 31 of 121) and a rate of 5.3% for HGV RNA (5 of 94) in venous blood (Fig 1). The prevalence of TTV DNA appeared in all age groups and there was no significant difference between the sexes. Co-infection of HGV RNA and TTV DNA was found in one case.

DISCUSSION

This cross-sectional survey investigated the seroprevalence of viral hepatitis infection

in Hmong people who live in the highlands of northern Thailand. Hmong traditionally lived in remote highland villages, but the development of extensive highland road networks, an emphasis on cash crops for lowland markets, and increasing migration to lowland urban centers for education and employment has brought Thai Hmong, and other formerly isolated highland minority peoples, into close contact with the majority ethnic Thai population.

Improvements in sanitation and hygiene in developing countries have been associated with a reduced prevalence of HAV infection in some population groups, especially children and adolescent (Poovoravan *et al,* 1997; Kunasol *et al*, 1998). A shift of epidemiology in the young resulted in increased susceptibility to HAV infection and an outbreak of HAV among schoolchildren in Southern Thailand (Sinlaparatsamee *et al*, 1995).

In this study, most of the Hmong infected with HAV appear to have acquired their infection after the age of ten. This implies that Hmong are exposed to HAV from early childhood. In general, there is less severity of HAV infection at younger ages. Females in this population are at significantly higher risk of HAV infection than males.

Previous reports show that the prevalence of HEV infection varies from 1.8-6.2% in different regions of Thailand (Poovorawan *et al*, 1996). Although a high prevalence of HAV was demonstrated in this Hmong population, the evidence of HEV infection in both male and female was still limited, with an overall seroprevalence rate of 6.5%. These data suggest that HAV is more widespread than HEV in this population.

HBV infection in Thailand is at an intermediate endemic level, with the 8-10% HBV carriage in males and 6-8% in females (Merican *et al*, 2000). In this study, the frequency of HBV infection was approximately 70%. Interestingly, high carriage rates for HBsAg antigenemia (13.8%) were found; males (20%) were more affected than females (10%). Pos-

sible explanations for of HBV transmission were a family history of hepatitis and host genetic factors rather than risk behaviors such as tattooing and injecting drugs. A previous study of multiethnic subjects in northern Thailand showed differences in the rates of HBV infection by tribe: from a low of 4.7% among the Akha to a high of 22.6% among the Lahu people (Ishida *et al*, 2002). A high rate of HBV carriage in the Hmong population increases the risk of chronic liver disease, cirrhosis and hepatocellular carcinoma. A mass hepatitis B vaccination program in healthy children and adolescents in Taiwan resulted in a significant reduction of HBV carriage rate (Ni *et al*, 2001). Consideration should be given to extending the successful EPI vaccination program to formerly remote populations such as the Hmong, otherwise such groups will continue to be reservoirs for HBV transmission and hepatitis B carriage will not be eradicated.

A high prevalence of immunity to HCV (90%) and HDV (60%) has been reported amongst Thai IVDUs, but not in the general population (Louisirirotchanakul *et al*, 1988; Wasi *et al*, 1990; Luksamijarulkul and Puchtaweesak, 1996). The principal occupation of Hmong people in Thailand is the intensive cultivation of fruits and vegetables. None of the individuals in this study reported that they were intravenous drug users and, therefore, it is not surprising that the prevalence of HCV infection was relatively low (2%) - similar to the prevalence in the general population (Wasi *et al*, 1990; Ishida *et al*, 2002). HDV was also found in one subject with HBV antigenemia.

Infection with two novel viruses associated with transfusion was also found. A high prevalence of TTV DNA has been reported in people in Thailand with chronic infections and among Thai blood donors (Tanaka *et al*, 1998); a high prevalence of TTV DNA was found in this study. The frequency of HGV was low and similar to the prevalence of this virus among blood donors throughout the world (range 0.9%-10%) (Cheung *et al*, 1997). No significant differences by age and sex were noted in this study. The roles of HGV and TTV in hepatitis

remain uncertain.

In summary, the Hmong population is at high risk from HAV and HBV infection but not from the other types of viral hepatitis. Vaccination against HAV in the Hmong population may be unnecessary because the infection usually occurs in early childhood when it causes asymptomatic disease. However, because of the high rate of carriage of HBV, a massive HBV vaccination program should be considered.

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