Short Communication

Seroprevalence and Subtype Distribution of Hepatitis C Virus among Blood Donors and Intravenous Drug Users in Northern/Northeastern Thailand

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SUMMARY: The prevalence of antibodies against hepatitis C virus (HCV) and the distribution pattern of HCV subtypes were analyzed among healthy blood donors and intravenous drug users (IVDUs) in northern/northeastern Thailand. The prevalence of anti-HCV antibodies was 3.2% (26/820) among blood donors in Khon Kaen, while it was 90% (71/79) among IVDUs in Chiang Rai. HCV RNA was detected in all anti-HCV-positive sera collected from blood donors and IVDUs tested, as determined by reverse transcription-PCR analysis. Sequence analyses of amplified fragments of the HCV genome revealed that in Khon Kaen and Chiang Rai, Thailand, HCV-3a (50-60%) was the most common HCV subtype, followed by HCV-1a, HCV-1b, and subtypes of clade 6, each at 10-20%.

Hepatitis C virus (HCV) is the major etiologic agent of post-transfusion hepatitis as well as sporadic non-A, non-B hepatitis worldwide (1). In general, HCV causes only mild clinical symptoms, if any, at the acute phase of infection. After more than 10 years of viral persistence, however, HCV infection often leads to liver cirrhosis and hepatocellular carcinoma (1). HCV is currently classified into six clades (formerly called genotypes), each of which can be further divided into a number of subtypes (2, 3). The prevalence of each HCV subtype varies according to geographical region and, more importantly, hepatopathogenicity and response to interferon treatment are thought to vary with different HCV subtypes (1). We previously reported the prevalence of HCV infection and HCV subtype distribution among various populations-such as blood donors, intravenous drug users (IVDUs), and patients with chronic liver disease-in Chiang Mai, Thailand (2, 4). In the present study, we performed a similar survey in other cities in northern/northeastern Thailand.

Eight-hundred-and-twenty serum samples were collected from healthy blood donors (mean age, 28.0 years; range, 17-60 years; male:female = 2.5:1) at the Blood Transfusion Center, Faculty of Medicine, Khon Kaen University, Khon Kaen, and 79 serum samples were obtained from IVDUs (mean age, 35.5 years; range, 21-55 years; male:female = 5.6:1) at Mae Chan Hospital, Chiang Rai, Thailand, in 1996. The sera were tested for anti-HCV antibodies using second-generation ELISA (Abbott Diagnostics, Wiesbaden-Delkenheim, Germany). Anti-HCV-positive sera were further analyzed for HCV RNA by reverse transcription-PCR that was designed to amplify a portion of the NS5B region or 5'-untranslated region (5'UTR) of the viral genome. When NS5B region sequences were successfully amplified, HCV subtypes were determined by

sequence analysis, as described previously (2, 4, 5). In brief, RNA was extracted from anti-HCV-positive sera using TRIzol reagent (Life Technologies, Gaithersburg, Md.) and was reverse transcribed into cDNA using an HCV specific primer, 167R, for the NS5B region of the HCV genome. The resultant cDNA was amplified by nested PCR by using various sets of primers in order to increase amplification efficiency. Amplified DNA fragments were sequenced by a direct sequencing method using a *Taq* DyeDeoxy Terminator Cycle Sequencing kit and an ABI 373A DNA sequencer (Applied Biosystems, Foster City, Calif.). Each sequence obtained was compared with the reported sequences of known subtypes, and each isolate was assigned a subtype based on the sequence similarity. Also, to confirm the subtype classification, especially with isolates of clade 6, a phylogenetic tree was constructed using a 6-parameter neighbor-joining method, as described previously (2, 5). In the case of unsuccessful amplification of the NS5B region, 5'UTR sequences were targeted for amplification using 5'UTR-specific primers. When 5'UTR sequences were obtained, each isolate was assigned a clade, but not a subtype, based on the sequence similarity.

The prevalence of anti-HCV antibodies among healthy blood donors in Khon Kaen was 3.2% (26/820) (Table 1), which was comparable with that observed in Chiang Mai, another city in northern Thailand (4). On the other hand, 71 (90%) of 79 sera obtained from IVDUs in Chiang Rai were positive for anti-HCV antibodies (Table 1). An extremely high prevalence of anti-HCV antibodies among IVDUs was observed also in Chiang Mai, with a positivity of 97% (4).

Anti-HCV-positive sera were then tested for HCV RNA. A portion of the NS5B region of the HCV genome could be amplified from 25 (96%) of 26 anti-HCV-positive blood donor sera, and from 9 (90%) of 10 anti-HCV-positive IVDU sera tested. The remaining two sera that were negative for NS5B amplification, one from a blood donor and the other from an

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Table 1. Prevalence of anti-HCV antibodies and distribution pattern of HCV subtypes among blood donors and IVDUs in northern/northeastern Thailand

Group	Prevalence of anti-HCV (%)	Relative prevalence of HCV subtype (%)					
		Clade 1		Clade 3		Clade 6 ^a	Clade 4 ^b
		1a	1b	3a	UC ^c		
Blood donor	26/820 (3.2)	3/26 (12)	4/26 (15)	16/26 (62)		2/26 (8)	1/26 (4)
IVDU	71/79 (90)	2/10 (20)	0/10 (0)	5/10 (50)	1/10 (10)	2/10 (20)	0/10 (0)

^aDifferent HCV clade 6 subtypes are included.

^bDetermined based on the 5'UTR sequences.

^cDetermined based on the 5'UTR sequences. Subtype unclassified.

IVDU, were subjected to amplification for a portion of the 5'UTR sequence and were found to be positive. Thus, HCV RNA was detected in all of the anti-HCV-positive sera tested in this study. Nucleotide sequences of the amplified fragments were determined and compared with the reported sequences. Based on the sequence similarity in the NS5B region with the sequences of known subtypes, each HCV isolate was assigned a subtype, such as HCV-1a, -1b, and -3a. Among healthy blood donors in Khon Kaen, HCV-3a was most common, accounting for 62%, followed by HCV-1b (15%) and HCV-1a (12%) (Table 1). Two isolates from blood donors (8%) showed considerable degrees of sequence diversity when compared with known HCV subtypes, as has been reported with the isolates of clade 6 (2, 5), and it was rather difficult to determine conclusively their phylogenetic positions based only on the percent of similarity. Therefore, phylogenetic analysis was performed to classify those isolates more precisely. The result has demonstrated that they belong to HCV clade 6 (Fig. 1). Since standard nomenclature system for the subtypes of clade 6 has not been established except for 6a (3), no particular subtype was assigned to them. The remaining one isolate (4%) from blood donors, which was negative for NS5B but positive for 5'UTR, was classified into clade 4 based on its 5'UTR sequence (2, 5). As for 10 isolates from IVDU, 9 isolates were each assigned a subtype based on NS5B sequences; 5 were isolates of HCV-3a, and two each were isolates of HCV-1a and clade 6. The remaining one, which was negative for

NS5B amplification, had a 5'UTR sequence that completely matched the representative sequence for HCV-3a and, therefore, was tentatively assigned an unclassified subtype of clade 3. Thus, among IVDUs, too, HCV-3a was most common (50-60%), followed by HCV-1a (20%) and subtypes of clade 6 (20%).

Our present study showed a relatively high prevalence of HCV infection among blood donors (3.2%) in Khon Kaen, Thailand, which was approximately the same as that observed in Indonesia and the Philippines but 2-3 times higher than that observed in Japan. Also, our study showed an extremely high prevalence among IVDUs (90%) in Chiang Rai, Thailand, with the results being comparable with those obtained in other areas of Thailand (2, 4, 6). Therefore, screening blood samples for anti-HCV antibodies, as well as other precautions, should be strongly recommended for this country as well. Currently, in Thailand, HCV screening at blood transfusion centers is performed only in big hospitals and in some provincial hospitals, but not always in small hospitals mainly due to economic reasons.

HCV-1b is the most common HCV subtype in East Asia and in some European countries, while HCV-1a is most common in the United States. HCV-3a was first identified in Thailand but was later reported to be common in the United Kingdom, Germany, and Brazil. On the other hand, HCV-4a is commonly found in the Middle East and HCV-5a was reportedly confined to South Africa. HCV clade 6, which can be further

Fig. 1. Phylogenetic tree for HCV NS5B sequences. Only the representative strains of each subtype in each clade (3) and the Thai isolates obtained in the present study (boldface letters) are shown, with bootstrap values along the tree. Clades are indicated by numerals along the tree and subtype names are shown in parentheses. DDBJ/EMBL/GenBank accession numbers are: EUHK2 (Y12083), JK046 (D63822), Th846 (AB027610), Th553 (AB027608), Th555 (AB027609), HCV-J (D90208), HCV-1 (M62321), HC-G9 (D14853), EUH1480 (Y13184), HC-J6 (D00944), HC-J8 (D10988), ED43 (Y11604), NZL1 (D17763), HCV-Tr (D49374), and JK049 (D63821).

divided into more than a dozen of subtypes, is found almost exclusively in the southeastern part of the Asian continent, including Hong Kong, Vietnam, and Thailand (2). We previously reported that in Chiang Mai HCV-3a is prevalent among anti-HCV-positive healthy blood donors, IVDUs and patients with chronic liver disease, followed by HCV-1a, HCV-1b, HCV-3b, and new subtypes of clade 6 (2, 4). A similar pattern of HCV subtype prevalence was observed among blood donors and patients with chronic hepatitis in Bangkok, in the central part of Thailand (6, 7). On the other hand, HCV isolates of clade 6 were not commonly found in Hat Yai, a city in southern Thailand, although the distribution patterns of other subtypes were similar to those observed in Chiang Mai and Bangkok (8). Therefore, surveillance of other areas is still important to better understand HCV epidemiology in Thailand as a whole.

In this study we investigated HCV subtype distribution in areas other than Chiang Mai, Bangkok, and Hat Yai. Khon Kaen is located in the northeastern part of Thailand near its border with Laos. Chiang Rai is located in the northern part of Thailand near its border with Myanmar. The present study demonstrated that, as in Chiang Mai and Bangkok, HCV-3a was the most common HCV subtype, followed by HCV-1a, HCV-1b, and subtypes of clade 6 in Khon Kaen and Chiang Rai.

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