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Genotypic Distribution of Hepatitis C Virus in Different Regions of Thailand

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The genotypic distribution of hepatitis C virus (HCV) isolated from blood donors from four major regions of Thailand was studied by reverse hybridization assays. PCR-amplified products from the 5′ noncoding and core regions of the viral genome were hybridized to genotype- and subtype-specific probes which were immobilized on the nitrocellulose membrane. Of 332 anti-HCV-positive plasma samples studied, 71% contained HCV RNA. HCV genotype 3a was the most prevalent genotype (39%), followed by genotype 1b (20%) and genotype 6 group variants (18%). HCV genotype 1a was identified among 9% of all isolates. Other genotypes (genotype 1 which was neither 1a nor 1b, genotype 3b, and an unclassified genotype) were uncommon. There was no difference in the mean age of the donors infected with different HCV genotypes. The genotypic distribution pattern of HCV was similar among HCV isolates from different regions of Thailand.

Hepatitis C virus (HCV) is a major etiologic agent of transfusion-associated hepatitis. Chronic hepatitis occurs in more than 50% of HCV-infected patients and can lead to cirrhosis and liver cancer. The genetic variation of this virus has been demonstrated previously (for a review, see reference 3). Classification of HCV is based on the diversity of the genome, and the criterion for HCV classification was proposed by Simmonds and colleagues (19). According to this criterion, the virus could be classified into at least six major genotypes and 11 subtypes. Some genotypes are endemic worldwide, while others may be restricted to distinct geographical regions (13). The importance of HCV diversity has been demonstrated in several aspects. A study with chimpanzees showed that antibodies specific to an HCV subtype could not prevent superinfection with isolates of other genotypes (16). Individuals infected with genotype 2 or 3 tend to respond better to antiviral treatment than those infected with genotype 1b (8, 17). The diversity of the HCV genome also has implications for the development of diagnostic assays and an effective vaccine. The reactivity of immunology-based assays for the detection of anti-HCV antibodies may depend on the genotypes of viral antigens (12, 18).

The prevalence of antibodies to HCV in a Thai population that was studied was 1.5 to 5% (4, 21). An early study of the Thai isolates of HCV showed that genotype 3a was found in two of four HCV-infected patients; the other two were of genotype 3b (15). More recent studies demonstrated that HCV genotype 3a was the most prevalent genotype in Thai blood donors and patients and accounted for almost half of all isolates, whereas genotype 3b accounted for only 2 to 8% of all HCV isolates (6, 11). HCV genotype 1b was found in 22% of Thai blood samples, followed by genotype 1a (13%). However, the blood samples in these studies were mainly obtained from subjects in Bangkok, which is in central Thailand. It is not known if the pattern of genotypic distribution of isolates in other regions of the country is similar to that in the central region. Interestingly, three HCV isolates of a novel genotype were identified from northern Thailand; genotype assignment will depend on further nucleotide sequence data (1). It seems that the genotypic distributions of HCV isolates from different regions of Thailand may differ from one another. Recently, HCV genotype 6 group variants were identified in Thailand and neighboring countries such as Vietnam and Hong Kong (19, 23). These genotype 6 group variants could not be differentiated from genotype 1b isolates by assays based on 5′ noncoding region (5′-NCR) sequences, which were similar between isolates of these two genotypes. Therefore, there is a need for the development of new tool to study the viral genotype that could discriminate between these two genotypes. The importance of genotype 6 group variants in Thailand is not clearly known.

In order to analyze the pattern of genotypic distribution of HCV in different regions of Thailand, two reverse hybridization assays for HCV genotyping based on the 5′-NCR and the core region have been developed in this study. The HCV genotype and subtype was identified primarily by the 5′-NCR-based genotyping assay. Genotype 6 group variants were then subsequently differentiated from genotype 1b isolates by using the core region-based genotyping assay. These assays were used for studying the genotypic distributions of HCV isolates in different regions of Thailand. In addition, the correlation between certain HCV genotypes and degrees of liver injury was investigated.

MATERIALS AND METHODS

Samples. A total of 332 anti-HCV-positive serum and plasma samples were collected from Blood Transfusion Centers at Siriraj Hospital (central Thailand; 191 samples), Khonkaen Regional Hospital (northeastern Thailand; 97 samples), Songkhla Hospital (southern Thailand; 38 samples), and Chiangmai Hospital (northern Thailand; 6 samples). Self-exclusion criteria were applied to these donors prior to blood donation, and none had a previous history of hepatitis. The antibodies to HCV were assayed by one of the second-generation enzyme immunoassays (EIAs) manufactured by either Abbott (North Chicago, Ill.), Organon (United Biomedical, Buffalo, N.Y.), Murex (Dartford, England), or Diagnostic Biotechnology (Singapore). Seropositivity was confirmed by using EIAs from other manufacturers that differed from those used for the initial screening. In addition, samples from Khonkaen were also tested for anti-HCV antibodies by particle agglutination assay (Serodia, Fujirebio, Japan), and the results were confirmed by EIA (Organon). Plasma samples were aliquoted into 1.5-ml vials and were immediately stored at −70°C. Serum samples from blood donors at Siriraj Hospital were also tested for alanine aminotransferase (ALT) levels. For 16 samples included in this study the nucleotide sequences within the 5′-NCR

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TABLE 1. Nucleotide sequences of primers and probes used in this study

Primer or probe	Nucleotide sequence (5′→3′)	Specificity to HCV genotype	Position[a]	Polarity
A1L[b, c]	CACTCCCCTGTGAGGAACTACTGT	Universal	−304– −281	+
L149[c]	CTHGTYGCGCGCACACCCA	Universal	131–149	−
A18R[b]	GCACTCGCAAGCACCCTATC	Universal	−47– −28	−
BA13L[b, c]	Biotin-CTGTCTTCACGCAGAAAGC	Universal	−284– −266	+
BA16R[b]	Biotin-CCCTATCAGGCAGTACCAC	Universal	−59– −41	−
BA18R[c]	Biotin-CCAANCKNGGGCCCCTGCGC	Universal	114–133	−
Probe A	AGCCGAGTAGYGTTGGG	Universal	−90– −74	+
Probe B	CGGTCGTCCTGGCAATTC	1	−171– −154	−
Probe C	CTAGCRRTCTTGCGGG	1a	−104– −89	−
Probe D	CTAGCAGTCTCGCGGG	1b/6 group variants	−104– −89	−
Probe E	GGATAAACCCACTCTATG	2	−142– −125	+
Probe F	CGGTCACCCCAGCGATT	3a	−170– −154	−
Probe G	GGTCATCCCGGCGATT	3b	−170– −155	−
Probe H	ATGGGATCCCTGGATGCTGGGTCTTCCAAA	Negative control	Specific to HBV sequence	
Probe I	Biotin-CTGTCTTCACGCAGAAAGC	Positive control	−284– −266	−
Probe J	GCACRAATCCTAAACCTC	1b	5–22	+
Probe K	GCACRCTTCCWAAACCYC	6 group variants	5–22	+

[a] Position numbering is according to Choo et al. (5).
[b] Primers used in the 5′-NCR-based genotyping assay: outer primers A1L and A18R and inner primers BA13L and BA16R.
[c] Primers used in the core region-based genotyping assay: outer primers A1L and L149 and inner primers BA13L and BA18R.

and either the nonstructural 5 (NS5) or the envelope 1 (E1) regions were also determined. Phylogenetic analysis was applied to these sequences, and genotypes were determined from the phylogenetic tree. The mean ± standard deviation age of these donors was 29.0 ± 8.4 years (age range, 16 to 51 years).

RNA extraction and RT-PCR. RNA was extracted by a modified acid guanidinium thiocyanate-phenol-chloroform extraction method as described previously (20). A reverse transcription (RT)-PCR (RT-PCR) assay was performed in a single tube by using two outer primers. The RT process was carried out at 42°C for 30 min, and then the reaction was subjected to 35 cycles of PCR; each cycle consisted of 94°C for 1 min, 50°C (40°C for the core region-based assay) for 1 min, and 72°C for 2 min, with a final extension step at 72°C for 10 min. The first-round PCR product was amplified under the same conditions, but with the inner set of primers. The primers used in the second-round PCR were biotinylated at their 5′ ends. The nucleotide sequences of the primers used in this study are presented in Table 1. The amplification products were detected by electrophoresis in a 2% agarose gel, stained with ethidium bromide, and visualized under UV light. Strict laboratory precautions were exercised to prevent any contamination and carryover (10).

Validation of reverse hybridization assays. Reverse hybridization assays were based on the complementary binding of genotype- and subtype-specific probes to the PCR-amplified product. The oligonucleotide probes were derived from the nucleotide sequences within the 5′-NCR and the core region of the Thai isolates and those deposited in GenBank database and are presented in Table 1. The 5′-NCR-based genotyping assay contained 6 HCV genotyping probes, each specific to HCV genotype 1, 1a, 1b/6 group variants, 2, 3a, and 3b. A universal probe reactive to all of the HCV genotypes known to date was also included. The core region-based genotyping assay consisted of two HCV genotyping probes; one could bind to genotype 1b but not genotype 6 group variants (probe J) and the other could bind to genotype 6 group variants but not to genotype 1b (probe K). The oligonucleotide probes were tailed with dTTP by using a terminal transferase enzyme (Promega, Madison, Wis.) to allow for the attachment of the tailed probes to the nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) (9). Two picomoles of each probe, except for 1 pmol each of probes H and I, was immobilized onto the membrane by using a slot blot apparatus (Hybri-Slot Manifold; Bethesda Research Laboratories, Gaithersburg, Md.). Ten microliters of the second-round amplification product was denatured in 0.4 M NaOH–10 mM EDTA solution for 10 min at room temperature. The membrane was prehybridized for 5 min at room temperature in buffer containing 3 M tetramethylammonium chloride (TMAC), 50 mM sodium phosphate (pH 6.8), 10 mM EDTA (pH 8.0), 5× Denhardt's reagent (0.1% bovine serum albumin, 0.1% Ficoll, and 0.1% polyvinylpyrrolidone), and 0.6% sodium dodecyl sulfate (SDS). After denaturation, 1 ml of fresh buffer was added to the amplification product, and the mixture was transferred to the membrane. Hybridization was carried out at 48°C for 2 h. The membrane was washed three times in washing buffer (3 M TMAC, 0.2% SDS, 50 mM Tris-HCl [pH 8.0]), twice at room temperature for 5 min each time (low stringency), and once at 52°C for 30 min (high stringency). The membrane was incubated with peroxidase-labelled streptavidin (dilution, 1:2,000) (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) for 30 min at room temperature and then with 4-chloro-1-naphthol (Sigma, St. Louis, Mo.) for 10 min. For the 5′-NCR-based genotyping assay, the amplification products from HCV subtypes 1a, 1b, 3a, and 3b hybridize to probes C, D, F, and G, respectively. HCV subtypes 1a and 1b also bind to probe B, which is specific to HCV genotype 1 of any subtype. Other subtypes of HCV genotype 1 such as subtypes 1c and 1d also bind to probe B, but not to probe C or D. Genotype 6 group variants bind to probes A, B, D, and I, similar to genotype 1b, and variants with this pattern of hybridization are regarded as genotype 1b/6 group variants. In this system, probes specific to HCV genotypes 4 and 5 or other subtypes of genotypes 1, 2, and 3 were not included in the assay because these genotypes were not discovered in the nucleotide sequence study of Thai samples. It should be noted that there are many subtypes of type 3, some of which may resemble type 3a or 3b in the 5′-NCR; the 5′-NCR-based assay therefore cannot unequivocally identify all of the type 3 subtypes because other subtypes of type 3 may be identified as subtype 3a or 3b. The HCV universal probe (probe A) was designed to hybridize to all subtypes and genotypes of HCV known to date. Probe A could detect the presence of HCV in a sample and could also detect other HCV subtypes for which type-specific oligonucleotide probes were not included in this assay. The biotinylated probe (probe I) was included in this assay as a positive control for the enzymatic reaction. Probe H, a hepatitis B virus (HBV)-specific probe, represented a nucleotide sequence irrelevant to the HCV genome and thus was used as a negative control probe. An example of the results of the 5′-NCR-based and core region-based genotyping assays results are shown in Fig. 1.

For the core region-based genotyping assay, probe J could bind to genotype 1b but not to genotype 6 group variants, and the samples that could hybridize to this probe would be read as genotype 1b. Probe K could hybridize to genotype 6 group variants, and samples that were reactive to this probe would be interpreted as genotype 6 group variants. It should be noted that these two probes were not specific only to genotype 1b or genotype 6 group variants. Other HCV genotypes such as genotype 3a or 3b could also bind to one of these probes. Therefore, the core region-based genotyping assay was applied to samples with the genotype 1b/6 group variant pattern by the 5′-NCR-based assay to differentiate between HCV genotype 1b and 6 group variants.

Statistical analysis. Statistical analysis was performed by using the multifactorial analysis component of Statview, version 4.1, software (Abacus Concepts, Berkeley, Calif.). A difference was considered significant if the P value was ≤0.05.

RESULTS

Detection of HCV RNA in anti-HCV-positive samples. The results of HCV RNA detection are presented in Table 2. In summary, the rate of detection of HCV RNA in anti-HCV-positive blood donors in Thailand was 71.1%. The HCV RNA detection rates in anti-HCV-positive samples among the four major blood transfusion centers in Thailand were not statistically different (P = 0.16).

Reverse hybridization assays. The HCV genotype was determined from the pattern of hybridization to HCV genotype- and subtype-specific probes. The accuracy of the 5′-NCR-based genotyping assay was tested with 16 samples from HCV-in-

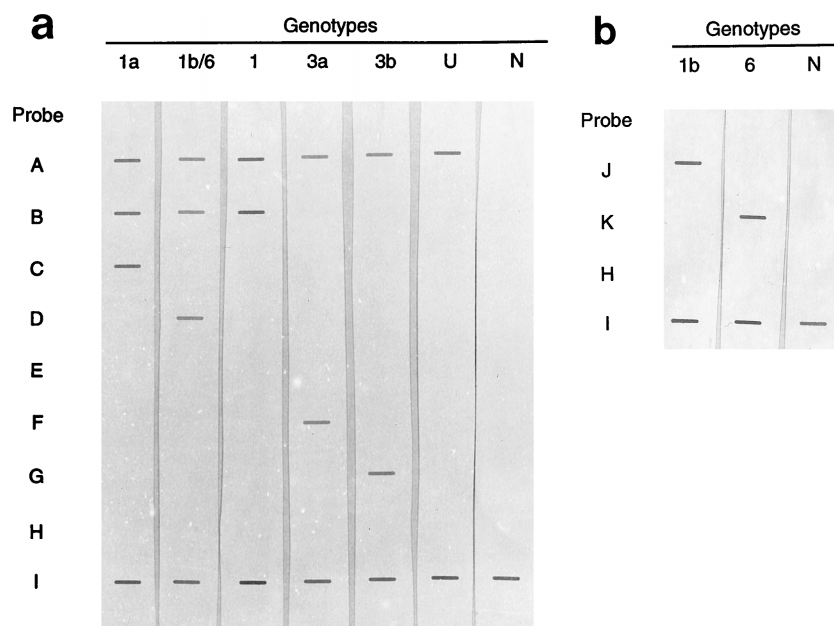

FIG. 1. Example of hybridization patterns of each genotype obtained from genotyping assays. (a) 5′-NCR-based genotyping assay; (b) core region-based genotyping assay.

fected Thai blood donors. The viral genome in these 16 samples was sequenced, and the genotype and subtype were determined by phylogenetic analysis (19a). All three samples with isolates belonging to HCV genotype 1a were positive with probes A, B, C, and I, but were negative with the remaining probes. All three samples with isolates belonging to HCV genotype 1b tested positive with probes A, B, D, and I, and all eight samples with isolates of HCV genotype 3a were positive with probes A, F, and I. Two samples with genotype 6 group variants had hybridization patterns similar to that of genotype 1b isolates. Comparison of the nucleotide sequences of these two isolates with that of probe D showed that the sequences were identical, and thus, samples with these variants would give the genotype 1b/6 group variant pattern by the 5′-NCR-based assay.

The core region-based genotyping assay was based on the difference in nucleotide sequence within the core region of the HCV genome between genotype 1b and genotype 6 group variants. The nucleotide sequences of genotype 6 group variants showed nucleotides CT at positions 10 and 11, instead of nucleotides AA at the same positions in genotype 1b. This assay was verified by using five samples, for which the 5′-NCR-based genotyping assay gave the genotype 1b/6 group variant pattern and for which the isolates' nucleotide sequences have been determined. Two samples contained genotype 6 group variants, and three samples contained isolates of genotype 1b. The results were as predicted. Therefore, this new assay was applied to differentiate between HCV genotype 1b and genotype 6 group variants. The HCV genotypes determined by this assay were divided into four categories: unclassified, genotype 1b, genotype 6 group variants, and mixed genotype 1b/6 group variants. The unclassified genotype meant that the HCV genome could not be amplified by the nested primers used in this assay, despite the presence of viremic status, as detected by PCR amplification with other sets of primers (data not shown). The mixed genotype 1b/6 group variants showed reactivities to both probes.

HCV genotypic distribution in Thai blood donors. The HCV genotypes of isolates in 230 HCV RNA-positive samples were determined by the 5′-NCR-based genotyping assay; 131, 71, 24, and 4 samples were from the central, northeastern, southern, and northern regions of Thailand, respectively. The results are presented in Table 3. All PCR-positive samples tested positive with the biotin-labelled primers used in the 5′-NCR-based assay (n = 230). Of those samples, 219 (95.2%) samples were

TABLE 2. Detection of HCV RNA in anti-HCV-positive samples from different blood transfusion centers in Thailand

Region of Thailand	No. (%) of samples	
	All samples tested	HCV RNA positive
Central	191	131 (68.6)
Northeastern	97	77 (79.4)
Southern	38	24 (63.2)
Northern	6	4 (66.7)
Total	332	236 (71.1)

TABLE 3. Distribution of HCV genotypes in different regions of Thailand determined by 5′-NCR-based genotyping assay

Genotype	No. (%) of samples				
	Central	North-eastern	Southern	Northern	Total
1a	13 (10.0)	5 (7.0)	3 (12.5)	0	21 (9.2)
1b/6 group variants	49 (37.4)	30 (42.3)	10 (41.6)	1	90 (39.1)
1 (non-1a, non-1b)	6 (4.6)	2 (2.8)	1 (4.2)	0	9 (3.9)
3a	51 (38.9)	30 (42.3)	7 (29.2)	2	90 (39.1)
3b	5 (3.8)	2 (2.8)	1 (4.2)	1	9 (3.9)
Unclassified	7 (5.3)	2 (2.8)	2 (8.3)	0	11 (4.8)
Total	131	71	24	4	230

TABLE 4. Distribution of HCV genotypes in central and northeastern regions of Thailand as determined by core region-based genotyping assay

5′-NCR-based genotype	No. of samples with the following core region assay-based genotype:				
	1	6 group variants	1 and 6 group variants	Unclassified	Total
1a	17	0	0	1	18
1b/6 group variants	40	26	1	12	79
1 (non-1a, non-1b)	1	5	0	2	8
Unclassified	0	5	0	1	6

typeable. The PCR amplification in the core region-based assay was less sensitive than that in the 5′-NCR-based genotyping assay. Of 111 samples studied, 95 (85.6%) samples tested positive with primers used in the core region-based assay, and all of those were typeable.

The genotypic distribution of HCV isolates among the different regions of Thailand tested was not significantly different ($P = 0.86$; Table 3). HCV genotype 3a and 1b/6 group variants were the most predominant subtypes found in Thailand, with prevalences of about 40% each. About 9% of the HCV isolates were found to be genotype 1a. Other subtypes of HCV genotype 1 were uncommon. Genotype 3b was less prevalent in this area and accounted for only 3.9% of the isolates. In this study group, genotypes could not be identified for 11 HCV isolates (4.8%). It was not known whether these isolates belonged to a new genotype and subtype or whether they were variants of the existing genotypes in Thailand.

HCV genotype 6 group variants in Thai blood donors. The core region-based genotyping assay was applied to 111 samples from the central and northeastern blood centers in Thailand. The patterns of these samples by the 5′-NCR-based genotyping assay were either genotype 1a, genotype 1b/6 group variants, genotype 1, or isolates with an unclassified genotype. The results are presented in Table 4. Fifty-two percent of samples with genotype 1b/6 group variants were reactive to probe J, 33% were reactive to probe K, and 15% were unclassified. One sample with genotype 1b/6 group variants was reactive to both probes J and K. Of the samples with the genotype 1 pattern (non-genotype 1a and non-genotype 1b) and the unclassified genotype, 71% were retyped as genotype 6 group variants, and 21% of the samples were unclassified. In summary, HCV genotype 6 group variants were identified in 32% of 111 samples tested. The overall prevalence of genotype 6 group variants was 18% of all HCV isolates in Thailand. The distribution of HCV genotypes was not different when compared between two centers studied ($P = 0.78$).

ALT levels in and age of blood donors infected with different HCV genotypes. The levels of ALT, a marker of liver injury, in the sera of 85 blood donors from central Thailand were analyzed. A liver enzyme level was considered abnormal if the ALT level was greater than 1.5-fold of the upper limit of the normal level (>60 IU/liter). There was a difference in ALT levels between the HCV RNA-positive and the HCV RNA-negative group ($P = 0.004$). The ALT level associated with genotype 1b infection was higher than those associated with HCV genotype 1a, 3a, or 6 group variant infection ($P = 0.04$) (Table 5). The mean age of the donors infected with each HCV genotype (Table 5) was not significantly different from that of the other donors ($P = 0.78$), and the mean age of donors with HCV RNA in their blood was not different from that of donors negative by the HCV RNA assay ($P = 0.92$).

DISCUSSION

In this study, the distribution of HCV genotypes in the four main regions of Thailand was analyzed to provide a clearer picture of the situation of HCV infection in Thailand. The results showed that approximately 70% of anti-HCV-positive Thais are viremic. It is not known if the individuals who were HCV RNA negative but seropositive had recovered from HCV infection and had cleared HCV from their bodies or if they represented a group that had a low viral load beyond the detection limit of the HCV RNA assay. The other possibility was that these blood donors were infected with extreme variants of the existing HCV subtypes so that the primers used in the HCV RNA assay could not amplify these variants.

The 5′-NCR-based and core region-based genotyping assays were developed for identifying HCV genotypes and subtypes. The 5′-NCR-based genotyping assay could not discriminate between genotype 1b and genotype 6 group variants because of nucleotide sequence homology at the position of probe D. A preliminary survey by Mellor et al. (14) showed that about 8.0% of HCV samples from southeast Asia belonged to genotype 6 group variants. Because genotype 6 group variants were also reported from Vietnam and Thailand (14, 23, 24), the significant contribution of these variants in this region of the world needed further investigation. To date, there are various genotyping methods for HCV identification such as restriction enzyme-based genotyping methods or the line probe assay (6, 22). The principles of these assays were based on the differences in nucleotide sequences within the 5′-NCR, and therefore, these assays could not differentiate genotype 6 group variants from genotype 1b. Study of nucleotide sequences within other regions of the viral genome is needed to definitely identify the genotypes of samples with the genotype 1b/6 group variant pattern. Other HCV genotyping assays such as the Murex serotyping 1-6 assay is reported to be more powerful than molecular typing techniques in identifying genotypes 1 and 6 (2). However, a number of Thai samples failed to be typed by this assay (19a). Consequently, a new genotyping assay based on the nucleotide sequence within the core region of the HCV genome was developed in this study and was able to discriminate between genotype 6 group variants and genotype 1b. Both the 5′-NCR-based and the core region-based assays were validated by performing the reverse hybridization assays with 16 samples of known HCV subtypes. The subtypes identified by these assays were similar to those obtained by nucleotide sequence analysis.

According to the 5′-NCR-based genotyping assay, genotype 3a and 1b/6 group variants were equally prevalent and other genotypes were uncommon. The distribution pattern of HCV genotypes was slightly different from those found in other

TABLE 5. Mean age of blood donors and number of blood donors with elevated serum ALT levels.

Geneotype	No. of samples	Age (yr [mean ± SD])	No. of samples with elevated ALT level (>60 IU/liter)/total no. tested (%)
1	1	32.0	0/0
1a	11	31.0 ± 7.5	1/6 (16.7)
1b	21	28.3 ± 7.6	7/16 (43.7)
3a	46	28.4 ± 8.4	7/27 (25.9)
3b	5	28.2 ± 6.8	0/3
6 group variants	19	31.6 ± 8.5	1/14 (9.0)
Unclassified	10	30.1 ± 10.0	0/2
HCV RNA negative	45	28.9 ± 9.1	1/17 (6.4)

studies of Thai samples (6, 11). When the core region-based genotyping assay was applied to samples with the genotype 1b/6 group variant pattern, approximately half of the samples contained genotype 1b and the remaining half contained genotype 6 group variants. On the basis of this finding, the genotype 6 group variants were found among at least 15% of the Thai isolates of HCV and genotype 1b was found among no more than 20%. The prevalence of genotype 3a in this study was slightly lower than that in other reports (6, 11, 15). The discrepancy may be due to the choice of the subjects. The subjects in the present study were exclusively blood donors, in contrast to subjects who were blood donors and chronic hepatitis and hemophiliac patients in the other studies.

The distribution of the HCV genotype in Thailand was different from those found in other countries in southeast Asia including Singapore, Indonesia, and the Philippines (7). HCV genotypes 1a and 1b were the most prevalent genotypes found in those countries, whereas in Thailand, genotype 3a was the most common genotype. The difference in the genotypic distribution patterns may reflect the difference in transmission routes and the origin of infection. HCV genotype 1a was found in blood products from the United States, and individuals who received these products were found to be infected with genotype 1a. Genotype 1a was also the major genotype found in patients receiving multiple transfusions, while genotype 3a was found primarily in intravenous drug abusers (17). The blood donors in the present study had no previous history of intravenous drug abuse, had not received blood products, and were negative for anti-human immunodeficiency virus antibody (data not shown). Further investigation is needed to clarify the transmission route in this population.

The finding in previous reports that HCV genotype 1b caused more severe liver injury as determined by ALT levels was confirmed in the present study. However, the mean age of individuals infected with genotype 1b was similar to the mean age of those infected with genotype 3a ($P = 0.96$). This finding was in contrast to the previous finding in European countries that showed a correlation between genotype 3a with a younger age group and genotype 1b with an older age group (17). Furthermore, the present study provided the first evidence that the serum ALT level in patients infected with genotype 6 group variants, which were found almost exclusively in southeast Asia, was lower than that in patients infected with genotype 1b and was similar to that found in patients infected with other genotypes.

In summary, this study demonstrated that HCV genotype 3a, 1b, and 6 group variants are predominant in Thailand. The genotypic distributions of HCV isolates from the various regions of Thailand were similar.

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