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Differential Prevalence of Hepatitis C Virus Subtypes in Healthy Blood Donors, Patients on Maintenance Hemodialysis, and Patients with Hepatocellular Carcinoma in Surabaya, Indonesia

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Determination of the prevalence of liver disease caused by hepatitis C virus (HCV) of various genotypes helps provide an understanding of the virulences of these genotypes. Differences in the prevalences of these genotypes are known to exist in the various geographical regions of the world. Hence, we performed seroepidemiological and molecular epidemiological analyses of HCV in Surabaya, Indonesia. The prevalences of anti-HCV antibodies were 2.3, 76.3 and 64.7% in healthy blood donors, patients on maintenance hemodialysis, and patients with hepatocellular carcinoma (HCC), respectively. HCV-2a was the most common (52%) among the HCV clones obtained from blood donors; this was followed by HCV-1b (15%), HCV-1a (7%), and HCV-1d (7%), a unique Indonesian subtype. The high prevalence of HCV-2a in blood donors was further supported by serotyping analysis that could discriminate HCV type 2 (HCV-2a and -2b) from HCV type 1 (HCV-1a, -1b, and -1d). HCV-1a, -1b, and -1d were strongly associated with elevated serum alanine aminotransferase (ALT) levels in blood donors, suggesting a possibly more pathogenic feature of those subtypes than HCV-2a. In patients on maintenance hemodialysis, HCV-1a and -1b (each 31%) were among the most common subtypes, and in contrast to the case with blood donors, HCV-1a, -1b, and -1d were found in those with normal ALT as well as those with elevated ALT levels. Impaired immune responses of hemodialyzed patients might be responsible for the apparently decreased hepatocytic injury caused by infection with HCV type 1. In patients with HCC, HCV-1b (57%) was the most common; this was followed by HCV-1d (19%) and HCV-2a (5%). Subtype prevalence was not different between HCC patients with advanced liver cirrhosis and those without advanced cirrhosis.

Hepatitis C virus (HCV) has been known to be a major causative agent of chronic liver disease such as chronic hepatitis and liver cirrhosis, which often leads to hepatocellular carcinoma (HCC) (14). The genomic structure of HCV resembles, to some extent, that of flaviviruses and pestiviruses, and therefore, the virus is considered to represent a new genus of the family *Flaviviridae* (14). On the other hand, it was recently proposed that HCV should be classified into a new virus family, named *Hepciviridae*, because of its comparatively large sequence diversity from other members of the family *Flaviviridae* (24). The HCV genome of about 9.5 kb has a long open reading frame, flanked with 5′ and 3′ untranslated regions (UTRs), which encodes a polyprotein precursor consisting of about 3,010 to 3,030 amino acid residues. The polyprotein is cleaved by the host signal peptidase and two other virally encoded proteases to generate at least 10 viral proteins: 4 structural proteins such as the core protein, the E1 envelope glycoprotein, and two types of E2 envelope glycoproteins (types A and B), and 6 nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (6, 9, 19).

Considerable sequence variation has been observed with different HCV clones. On the basis of the sequence diversity, HCV is now classified into at least six major genotypes, each of which can be further divided into a number of subtypes (3, 4, 18, 25). The prevalence of each HCV subtype has been reported to vary in different geographical areas (3–5, 18, 25). It is still possible to identify sequence variants that could represent novel types or subtypes of HCV by performing extensive surveillance in previously overlooked areas. Viral pathogenicity and sensitivity to interferon treatment have been reported to vary with different subtypes (15, 21, 23).

We previously reported the prevalence of each subtype, including two novel subtypes, among HCV isolates obtained from patients with chronic hepatitis and liver cirrhosis in Surabaya, Indonesia (11–13). In order to better understand HCV infection in the same area and also to look into a possible association of certain subtypes with disease severity, we performed seroepidemiological and molecular epidemiological analyses of the virus for other groups. Here we report the differential prevalence of HCV subtypes in healthy blood donors, patients on maintenance hemodialysis, and patients with HCC in Surabaya. In addition, the significance of the differential prevalence is discussed.

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MATERIALS AND METHODS

Serum samples. Sera were obtained from 2,234 healthy blood donors at the Red Cross Blood Transfusion Center, Surabaya, 76 patients on maintenance hemodialysis, and 34 patients with HCC at the Dr. Soetomo Hospital, Faculty of Medicine, Airlangga University, Surabaya. The sera were tested for antibodies

against HCV by second-generation enzyme-linked immunosorbent assays (ELISAs; UBI HCV EIA, United Biologicals, Inc.; Ortho HCV Ab ELISA Test II, Ortho Diagnostics, Inc.). Serum alanine aminotransferase (ALT) levels were determined by using the Granutest ALAT (Merck, Darmstadt, Germany) according to the manufacturer's instructions. Normal ALT levels were ≤23 U/liter for men and ≤19 U/liter for women when tested at 25°C.

RT-PCR to detect HCV RNA in serum samples. Anti-HCV-positive sera were subjected to reverse transcription-PCR (RT-PCR) to detect HCV RNA, as described previously (1, 4, 5, 13, 16). Briefly, we performed RT-PCR for portions of NS5B region sequences using different sets of primers so that we could amplify the corresponding sequences from as many serum samples as possible. When NS5B region sequences could not be amplified by the RT-PCR described above, we then tried to amplify 5′ UTR sequences in order to check for the presence or absence of HCV RNA in the samples. These methods have been shown to be highly sensitive for the detection of HCV RNA in 90 to 100% of anti-HCV-positive sera from patients with chronic liver disease in Japan (5) and Indonesia (13) and in 80 to 90% of anti-HCV-positive sera from blood donors in Thailand (1) and the Philippines (16). The PCR products were electrophoresed in a 2% agarose gel containing ethidium bromide and were visualized by UV illumination.

Subtype analysis. The nucleotide sequences of the amplified NS5B regions were determined by the direct sequencing method with the Taq Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems, Inc.) and the ABI 373A DNA Sequencer (Applied Biosystems, Inc.). The sequences were compared with those of known HCV types or subtypes by using a computer program (GENETYX-MAC, version 7.3; Software Development Co., Ltd., Tokyo, Japan). By following the criteria suggested by Simmonds et al. (25), HCV clones showing at the nucleotide level sequence homology with any of the reported subtypes of more than 88% were assigned the corresponding subtype. We found a sequence variant in a blood donor sample (see below) which showed 98% homology with other Indonesian variants, Td-3/93 and Td-35/93 (13). Those variants have subsequently been classified into a new subtype of HCV type 3 by detailed phylogenetic analysis (see the phylogenetic trees in the report by Doi et al. [4]). In this study we have therefore tentatively assigned those variants a new subtype, subtype HCV-3g, named so that it follows subtype HCV-3f (29).

When a subtype assignment could not be done because of the lack of NS5B amplification, the nucleotide sequences of the 5′ UTR were determined and compared with the consensus sequence motifs for each of the major genotypes reported previously (17). When the sequence of an HCV clone completely matched the consensus motifs of a major genotype, the HCV clone was assigned the genotype, e.g., HCV type 1.

Serotype analysis. The serotypes of the HCV clones were determined on the basis of the serum antibody responses of the infected individuals against C14-1 and C14-2 recombinant peptides, which carry antigenic determinants specific for serotype 1 (corresponding to HCV type 1) and serotype 2 (corresponding to HCV type 2), respectively (26). ELISA kits for the detection of antibodies against those peptides were a product of International Reagents Corp. (Kobe, Japan) and were kindly supplied by M. Kohara, The Tokyo Metropolitan Institute of Medical Sciences. Serotype determination was done as described previously (26). A cutoff index value of more than 1.0 was considered a positive reaction. Samples with a C14-1/C14-2 cutoff index ratio higher than 2:1 were judged to be serotype 1, and those with a C14-2/C14-1 cutoff index ratio higher than 2:1 were judged to be serotype 2.

RESULTS

Prevalence of anti-HCV antibodies among healthy blood donors, patients on maintenance hemodialysis, and patients with HCC in Indonesia. Sera obtained from 2,234 healthy blood donors were divided into two groups on the basis of their ALT titers. Two hundred sixty serum samples showed elevated ALT titers (>23 U/liter), and the remaining 1,974 serum samples showed normal ALT titers (≤23 U/liter). Twenty-three (8.8%) of the 260 serum samples with elevated ALT titers were positive for anti-HCV antibodies, whereas 9 (1.4%) of the 646 serum samples randomly picked from the normal ALT group were positive by the same test (Table 1). It was estimated by calculation that a total of 28 serum samples would have been positive for anti-HCV antibodies among the 1,974 serum samples with normal ALT titers. Therefore, the total number of anti-HCV-positive individuals among the 2,234 blood donors was estimated to be 51, with the overall anti-HCV prevalence being 2.3%. The prevalence of anti-HCV positivity in blood donors with elevated ALT titers was significantly higher than that in those with normal ALT titers ($P < 0.05$), but it was significantly lower than that in patients on maintenance hemodialysis and patients with HCC ($P < 0.001$). The prevalence of anti-HCV antibodies among patients on maintenance hemodialysis was 76.3%, and in contrast to blood donors, there was no significant difference in anti-HCV prevalence between those with normal ALT titers and those with elevated ALT titers. A high prevalence of anti-HCV antibodies was also observed with HCC patients, whether or not the patients had advanced liver cirrhosis as diagnosed by ultrasonography.

TABLE 1. Prevalence of anti-HCV antibodies among blood donors, patients on maintenance hemodialysis, and patients with HCC in Surabaya

Group	No. positive/no. tested (%)
Blood donors[a]	
Normal ALT level	9/646 (1.4) (28/1,974)[b]
Elevated ALT level	23/260 (8.8)[c]
Total[b]	51/2,234 (2.3)
Hemodialysis patients	
Normal ALT level	31/40 (77.5)
Elevated ALT level	27/36 (75.0)
Total	58/76 (76.3)
HCC patients	
Without advanced liver cirrhosis[d]	14/22 (63.6)
With advanced liver cirrhosis	8/12 (66.7)
Total	22/34 (64.7)

[a] A total of 646 serum samples randomly picked from the 1,974 serum sample with normal ALT levels and all of the 260 serum samples with elevated ALT levels were tested.
[b] Estimated values obtained by calculation.
[c] $P < 0.05$ (χ^2 test with Yates' correction) compared with blood donors with normal ALT levels. $P < 0.001$ (χ^2 test with Yates' correction) compared with patients on maintenance hemodialysis or patients with HCC.
[d] Diagnosed by ultrasonography.

Detection of HCV RNA by RT-PCR for the NS5B and 5′ UTR in sera obtained from healthy blood donors, patients on maintenance hemodialysis, and patients with HCC. Anti-HCV-positive sera from 32 blood donors (31 males and 1 female; mean age, 42.3 years), 33 patients on maintenance hemodialysis (28 males and 5 females; mean age, 48.1 years), and 22 patients with HCC (16 males and 6 females; mean age, 59.3 years) were further analyzed for HCV RNA. Twenty-four (75%) of the 32 serum samples obtained from blood donors were positive for NS5B amplification with one or more of the different primer sets (Table 2). We did not retest the negative samples for NS5B amplification. The 8 serum samples that had been negative for NS5B amplification were subjected to RT-PCR for the 5′ UTR. Three of them became positive for the 5′ UTR, but the other five serum samples remained negative after two independent amplifications. In total, 27 (84%) of the 32 blood donor samples were positive for HCV RNA. Of the

TABLE 2. Positivity of HCV RNA in anti-HCV-positive sera from blood donors, patients on maintenance hemodialysis, and patients with HCC in Surabaya

Target region	No. positive/no. tested (%)		
	Blood donors	Hemodialysis patients	HCC patients
NS5B	24/32 (75)	25/33 (76)	18/22 (82)
5′ UTR	3/8[a]	7/8[a]	3/4[a]
Total	27/32 (84)	32/33 (97)	21/22 (95)

[a] Samples negative for the NS5B amplification were examined.

TABLE 3. Prevalence of each subtype among HCV clones obtained from anti-HCV-positive blood donors, patients on maintenance hemodialysis, and patients with HCC in Surabaya

Patient group	No. of samples	No. of HCV clones (%) with each subtype:							
		1a	1b	1d	2a	3g	Type 1[a]	Type 4[a]	UC[b]
Blood donors									
Normal ALT levels	8	0 (0)[c]	0 (0)[c]	0 (0)[c]	6 (75)	0 (0)	2 (25)	0 (0)	0 (0)
Elevated ALT levels	19	2 (11)[c]	4 (21)[c]	2 (11)[c]	8 (42)	1 (5)	1 (5)	0 (0)	1 (5)
Total	27	2 (7)	4 (15)	2 (7)	14 (52)[d]	1 (4)	3 (11)	0 (0)	1 (4)
Hemodialysis patients									
Normal ALT levels	16	6 (38)	3 (19)	1 (6)	1 (6)	0 (0)	5 (31)	0 (0)	0 (0)
Elevated ALT levels	16	4 (25)	7 (44)	0 (0)	2 (13)	0 (0)	3 (19)	0 (0)	0 (0)
Total	32	10 (31)[e]	10 (31)	1 (3)	3 (9)	0 (0)	8 (25)	0 (0)	0 (0)
HCC patients									
Without advanced cirrhosis[f]	13	0 (0)	8 (62)	2 (15)	0 (0)	0 (0)	3 (23)	0 (0)	0 (0)
With advanced cirrhosis	8	0 (0)	4 (50)	2 (25)	1 (13)	0 (0)	0 (0)	1 (13)	0 (0)
Total	21	0 (0)	12 (57)[g]	4 (19)	1 (5)	0 (0)	3 (14)	1 (5)	0 (0)

[a] On the basis of the 5′ UTR sequences.
[b] UC, unclassifiable because of ambiguous sequence results.
[c] Zero of 8 versus 8/19, $P < 0.05$ (Fisher's exact test; this test is more sensitive in demonstrating a significant difference when the number of the test samples is small).
[d] $P < 0.001$ and $P < 0.005$ (χ^2 test with Yates' correction) compared with patients on maintenance hemodialysis and patients with HCC, respectively.
[e] $P < 0.05$ (Fisher's exact test) and $P < 0.05$ (χ^2 test with Yates' correction) compared with blood donors and patients with HCC, respectively.
[f] Diagnosed by ultrasonography.
[g] $P < 0.01$ (χ^2 test with Yates' correction) compared with blood donors.

33 serum samples from patients on maintenance hemodialysis, 25 (76%) were positive for NS5B amplification and 7 others were positive for the 5′ UTR. In total, 32 (97%) of the 33 serum samples were determined to be positive for HCV RNA. Similarly, 18 (82%) of the 22 serum samples from HCC patients were positive for NS5B and 3 others were positive for the 5′ UTR, resulting in positive detection of HCV RNA in 21 (95%) of the 22 serum samples.

Prevalence of each subtype among HCV isolates obtained from healthy blood donors, patients on maintenance hemodialysis, and patients with HCC. Among 27 healthy blood donors, HCV-2a (52%) was found to be the most common and HCV-1b (15%) was the second most common on the basis of NS5B sequences; these were followed by HCV-1a (7%) and HCV-1d (7%) (Table 3). The prevalence of HCV-2a was significantly higher in blood donors than in patients on maintenance hemodialysis and patients with HCC ($P < 0.001$ and $P < 0.005$, respectively). In blood donors, 8 of 19 serum samples with elevated ALT titers but none of 8 serum samples with normal ALT titers were positive for either HCV-1a, -1b, or -1d. The difference between the two groups was statistically significant ($P < 0.05$). Thus, HCV-1a, -1b, and -1d were likely to be associated with elevations in serum ALT levels. An HCV variant was classified into HCV-3g. Three other clones (11%) were classified into HCV type 1 on the basis of the 5′ UTR sequences (17).

Among 32 patients on maintenance hemodialysis, HCV-1a and -1b (each 31%) were the most common. It should be noted that HCV-1a was significantly more common in patients on maintenance hemodialysis than in blood donors ($P < 0.05$) and patients with HCC ($P < 0.05$). However, the prevalence of HCV-1b was not significantly different between patients on maintenance hemodialysis and blood donors ($P = 0.120$; Fisher's exact test). Also, there was no significant difference in the subtype prevalence between hemodialyzed patients with normal ALT titers and those with elevated ALT titers (for HCV-1a, $P = 0.352$; for HCV-1b, $P = 0.126$; Fisher's exact test). Analysis of 5′ UTR sequences identified eight other clones (25%) of HCV type 1 in this group.

Among 21 patients with HCC, HCV-1b (57%) was the most common; this was followed by HCV-1d (19%) and HCV-2a (5%). The prevalence of HCV-1b was significantly higher in patients with HCC than in blood donors ($P < 0.01$). Analysis of 5′ UTR sequences identified three clones (14%) of HCV type 1 and a clone (5%) of HCV type 4.

Serotype analysis of HCV in blood donor samples. Nine of 14 serum samples that were found to contain HCV-2a by sequence analysis reacted to the C14-2 antigen, whereas 1 serum sample reacted very weakly to the C14-1 antigen and the remaining 4 serum samples reacted to neither antigen (Table 4). The latter four serum samples were shown to contain only low titers of anti-HCV antibodies, as determined by titration with a commercially available second-generation ELISA (data not shown). All of the sera containing HCV-1b and -1d were found to be serotype 1. The serotypes of two serum samples containing HCV-1a and two of three serum samples containing HCV type 1 (subtype unknown) were undetermined because of the lack of reactivity to the antigens. HCV-3g was classified

TABLE 4. Comparative study between subtypes and serotypes of HCV in sera obtained from blood donors in Surabaya

Subtype	Total no.	No. of samples determined to be:		
		Serotype 1	Serotype 2	UD[a]
1a	2			2
1b	4	4		
1d	2	2		
2a	14	1[b]	9	4
3g	1	1[b]		
Type 1[c]	3	1		2
UC[d]	1			1
Total	27	9	9	9

[a] UD, undetermined because of the lack of ELISA reactivity.
[b] Very weak reactivity.
[c] On the basis of the 5′ UTR sequences.
[d] UC, unclassifiable because of an ambiguous sequence result.

into serotype 1, but the reactivity was very weak. Overall, the concordance between the serotyping and the genotyping results was about 60% for the blood donors.

DISCUSSION

We have previously reported that HCV-1d is a unique subtype found only in Indonesia (11–13, 20). We also reported that HCV-1b and HCV-1d were prevalent in patients with chronic hepatitis and liver cirrhosis in Surabaya, Indonesia, representing more than 60% of the HCV clones obtained from the patients (13). Other investigators also observed a high prevalence of HCV-1b among chronic hepatitis patients in other areas of Indonesia (7, 8, 31). In the present study, we determined the subtype prevalence among HCV clones from healthy blood donors, patients on maintenance hemodialysis, and patients with HCC in Surabaya in order to better understand HCV infection in those patient groups and also to look into the possible association of certain subtypes with disease severity.

Unexpectedly, HCV-2a was the most common subtype among HCV clones from blood donors, representing 52% of the total (Table 3). Serotype analysis also showed a high prevalence of HCV type 2 in this group (Table 4), supporting the result of genotype analysis. One of the possible explanations for the high prevalence of HCV-2a in blood donors is that HCV-2a is less likely to cause clinical disease, which results from liver cell injury mediated either directly by a cytopathic effect or indirectly through immune mechanisms. The idea that HCV-2a is less pathogenic than other subtypes is in agreement with the observation that HCV-1b and -1d are associated with more severe liver damage than HCV-2a and -2b (13, 21, 23). We also observed in the present study that HCV-1a, -1b, and -1d are more strongly associated with elevations in serum ALT levels among blood donors and that the prevalence of HCV-2a is, accordingly, higher in blood donors with normal ALT levels than in those with elevated ALT levels (Table 3). Another possible explanation for the high prevalence of HCV-2a is that it has recently become more common among blood donors in this area than before, whereas HCV-1b was prevalent a few decades ago, when current patients with chronic liver disease had first contracted the virus infection. Such a changing pattern of HCV subtype prevalence over time has been reported for patients on maintenance hemodialysis and kidney recipients (22). If this is the case, the prevalence of HCV-2a among patients with chronic liver disease in this area will become higher in the future. In this connection, it should be noted that HCV-2a (35%) was prevalent in chronic liver disease patients in Yogyakarta, Indonesia, ranking as the second most prevalent subtype after HCV-1b (8). A possibility thus still remains that HCV-1b may not necessarily be more pathogenic than HCV-2a. Therefore, continued surveillance of the genotype prevalence among various populations over time as well as a long-term follow-up study of HCV-infected blood donors, especially those infected with HCV-2a, is needed to better elucidate the issue.

In patients on maintenance hemodialysis, HCV-1a and -1b were among the most common subtypes, each representing 31% of the total. Another 25% were identified as HCV type 1 on the basis of the 5′ UTR sequences. The high prevalence of HCV-1a in patients on maintenance hemodialysis is in sharp contrast to the subtype prevalence of other groups such as blood donors, patients with HCC (Table 3), and patients with chronic hepatitis or liver cirrhosis (13). This result is compatible with that observed with hemodialyzed patients in Yogyakarta (8) and suggests a possible increased risk for intraunit transmission of HCV infection among patients on maintenance hemodialysis. In this connection, it was reported that HCV-1a, which was uncommon among patients in Italy with community-acquired hepatitis, was highly prevalent in the same area among patients with hemophilia who apparently had contracted the virus infection through contaminated coagulation factor concentrates (21).

In contrast to the case with blood donors, HCV-1a, -1b, and -1d were frequently found in hemodialyzed patients with normal ALT levels, as well as in those with elevated ALT levels. It has been suggested that hepatocytic injury is mediated by cytotoxic T lymphocytes through an interaction with the Fas antigen expressed on HCV-infected hepatocytes (10). In general, patients on maintenance hemodialysis show impaired immune responses. It is reasonable, therefore, to assume that impaired cytotoxic T-lymphocyte responses fail to cause hepatocytic injury in some of the hemodialyzed patients infected with HCV-1a, -1b, or -1d, which otherwise could induce stronger cytotoxic T-lymphocyte responses to cause hepatocytic injury, as evidenced by elevated ALT levels. Further study of this matter is needed.

In patients with HCC, HCV-1b was the most common subtype; this was followed by HCV-1d (Table 3). These results are consistent with the previous results for patients with chronic hepatitis and liver cirrhosis (13). Subtype prevalence was not significantly different between HCC patients with advanced liver cirrhosis and those without advanced cirrhosis. However, the limitation in classifying the HCC patients into those with and those without advanced liver cirrhosis should be taken into consideration when interpreting the results, since the diagnosis of advanced liver cirrhosis was made on the basis of ultrasonography rather than liver biopsy. HCC is a consequence of the long-term persistence of HCV, and we do not know which subtype(s) was prevalent when the HCC patients were initially infected with the virus. Large-scale follow-up study of current blood donors in this area, among whom HCV-2a was the most prevalent, may give us a clue when looking into a possible relationship between HCC and a certain subtype(s) of HCV.

The NS5B sequence of an HCV variant obtained from a blood donor was 98% homologous at the nucleotide level with previously reported variants Td-3/93 and Td-35/93 (13). Those variants showed 70 to 74% homology with known subtypes of HCV type 3 (HCV-3a to -3f) (29). A sequence variant closely related to Td-3/93 and Td-35/93 was also found in India and was proposed to be a member of HCV type 3 (30). Our detailed phylogenetic analysis has confirmed this proposition, classifying the variants into a new subtype of HCV type 3 (4). Therefore, we assigned those variants HCV-3g in this study. The geographical distribution of this subtype needs to be determined by more extended surveillance. In this connection, Tokita et al. (27) reported the presence of HCV variants in Jakarta, Indonesia, which they assigned the 10th genetic group (HCV-10a) on the basis of phylogenetic analysis using the unweighted pair-wise grouping method. However, partial E1 and NS5B sequences of their variants showed >90% homology at the nucleotide level with those of Td-3/93 and Td-35/93, which have been assigned subtype HCV-3g, as described above. Similarly, previously proposed new HCV types 7, 8, and 9 (28) are considered to be new subtypes of HCV type 6 (4, 18). Thus, it has recently been suggested that HCV types 3 and 6 exhibit larger degrees of sequence diversity compared with other HCV types and that a more detailed phylogenetic analysis, like ours by the six-parameter neighbor-joining method, would be needed to determine the exact phylogenetic position(s) of newly identified sequence variants (4, 18). An inter-

national standard for HCV type or subtype classification is needed to avoid such confusion.

Serotype analysis with the C14-1 and C14-2 antigens is much cheaper and easier to perform than genotype analysis by RT-PCR and has been shown to be useful in discriminating between infections with HCV type 1 and those with HCV type 2 (26). Although it cannot discriminate subtypes of HCV type 1 or type 2 and also may not be applicable to infections with HCV type 3 or type 6 (2, 4), this serotyping method might still be advantageous in a large-scale epidemiological surveillance in areas where a vast majority of circulating HCV is either type 1 or type 2. Also, from the clinical point of view, it is important to discriminate HCV type 1 from type 2, since the former has been reported to be more pathogenic and more resistant to interferon treatment than the latter (15, 21, 23). In order to evaluate the efficacy of this serotyping method in Indonesia, we tried to determine the serotypes of HCV in Indonesian blood donors and to compare the results with those obtained by genotype analysis. The concordance between serotyping and genotyping results for blood donors was not very high, with the value being only about 60% (Table 4). In our previous experiments (2), we observed a much higher concordance with serum samples from chronic liver disease patients infected with HCV-1b, -1d, -2a, or -2b (data not shown). In general, the titers of anti-HCV antibodies are higher in patients with chronic liver disease than in blood donors. Therefore, the difference in the serotype/genotype concordance ratios between blood donors and liver disease patients given above is probably due to the sensitivity of the serotyping method. Improvement of the sensitivity is required to apply this serotyping system to epidemiological surveillance for blood donors.

In conclusion, we have shown the differential prevalence of HCV genotypes in blood donors with or without elevated ALT titers, patients on maintenance hemodialysis with or without elevated ALT titers, and patients with HCC with or without advanced liver cirrhosis. Although the number of samples tested was rather small, the results have raised a number of interesting implications regarding the HCV genotypes and the clinicopathological features of this virus infection and have given a rationale for the larger-scale study of the genotype prevalence in various populations.

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REFERENCES

1. **Apichartpiyakul, C., C. Chittivudikarn, H. Miyajima, M. Homma, and H. Hotta.** 1994. Analysis of hepatitis C virus isolates among healthy blood donors and drug addicts in Chiang Mai, Thailand. J. Clin. Microbiol. **32:** 2276–2279.
2. **Apichartpiyakul, C., H. Miyajima, H. Doi, M. Mizokami, M. Homma, and H. Hotta.** 1995. Frequent detection of hepatitis C virus subtypes 3a (HCV-3a) isolates in Thailand by PCR using subtype-specific primers. Microbiol. Immunol. **39:**285–289.
3. **Bukh, J., R. H. Miller, and R. H. Purcell.** 1995. Genetic heterogeneity of hepatitis C virus: quasispecies and genotypes. Semin. Liver Dis. **15:**41–63.
4. **Doi, H., C. Apichartpiyakul, K. Ohba, M. Mizokami, and H. Hotta.** 1996. Hepatitis C virus (HCV) subtype prevalence in Chiang Mai, Thailand, and identification of novel subtypes of HCV major type 6. J. Clin. Microbiol. **34:**569–574.
5. **Doi, H., S. Yoon, M. Homma, and H. Hotta.** 1994. Identification of hepatitis C virus subtype 3b (HCV-3b) among Japanese patients with liver diseases using highly efficient primers for reverse transcription-polymerase chain reaction. Microbiol. Immunol. **38:**159–163.
6. **Grakoui, A., D. W. McCourt, C. Wychowski, S. M. Feinstone, and C. M. Rice.** 1993. Characterization of the hepatitis C virus-encoded serine protease: determination of proteinase-dependent polyprotein cleavage sites. J. Virol. **67:**2832–2843.
7. **Greene, W. K., M. K. Cheong, V. Ng, and K. W. Yap.** 1995. Prevalence of hepatitis C virus sequence variants in southeast Asia. J. Gen. Virol. **76:**211–215.
8. **Hadiwandowo, S., F. Tsuda, H. Okamoto, H. Tokita, Y. Wang, T. Tanaka, Y. Miyakawa, and M. Mayumi.** 1994. Hepatitis B virus subtypes and hepatitis C virus genotypes in patients with chronic liver disease or on maintenance hemodialysis in Indonesia. J. Med. Virol. **43:**182–186.
9. **Hijikata, M., H. Mizushima, T. Akagi, S. Mori, N. Kakiuchi, N. Kato, T. Tanaka, K. Kimura, and K. Shimotohno.** 1993. Two distinct proteinase activities required for the processing of a putative nonstructural precursor protein of hepatitis C virus. J. Virol. **67:**4665–4675.
10. **Hiramatsu, N., N. Hayashi, K. Katayama, K. Mochizuki, Y. Kawanishi, A. Kasahara, H. Fusamoto, and T. Kamada.** 1994. Immunohistochemical detection of Fas antigen in liver tissue of patients with chronic hepatitis C. Hepatology **19:**1354–1359.
11. **Hotta, H., H. Doi, T. Hayashi, M. Purwanta, M. I. Lusida, W. Soemarto, and M. Homma.** 1994. Sequence analysis of hepatitis C virus obtained from Indonesian patients and identification of novel sequence variants, p. 310–313. *In* K. Nishioka, H. Suzuki, S. Mishiro, and T. Oda (ed.), Viral hepatitis and liver disease. Springer-Verlag, Tokyo.
12. **Hotta, H., H. Doi, T. Hayashi, M. Purwanta, W. Soemarto, M. Mizokami, K. Ohba, and M. Homma.** 1994. Analysis of the core and E1 envelope region sequences of a novel variant of hepatitis C virus obtained in Indonesia. Arch. Virol. **136:**53–62.
13. **Hotta, H., R. Handajani, M. I. Lusida, W. Soemarto, H. Doi, H. Miyajima, and M. Homma.** 1994. Subtype analysis of hepatitis C virus in Indonesia on the basis of NS5b region sequences. J. Clin. Microbiol. **32:**3049–3051.
14. **Houghton, M., A. Weiner, J. Han, G. Kuo, and Q.-L. Choo.** 1991. Molecular biology of the hepatitis C viruses: implications for diagnosis, development and control of viral disease. Hepatology **14:**381–388.
15. **Kanai, K., M. Kako, and H. Okamoto.** 1992. HCV genotypes in chronic hepatitis C and response to interferon. Lancet **339:**1543.
16. **Katayama, Y., N. G. Barzaga, A. Alipio, Soetjipto, H. Doi, S. Ishido, and H. Hotta.** 1996. Genotype analysis of hepatitis C virus among blood donors and inmates in Metro Manila, The Philippines. Microbiol. Immunol. **40:**525–529.
17. **Kleter, G. E. M., L.-J. van Doorn, J. T. Brouwer, S. W. Schalm, R. A. Heijtink, and W. G. V. Quint.** 1994. Sequence analysis of the 5′ untranslated region in isolates of at least four genotypes of hepatitis C virus in The Netherlands. J. Clin. Microbiol. **32:**306–310.
18. **Mellor, J., E. C. Holmes, L. M. Jarvis, P. L. Yap, P. Simmonds, and The International HCV Collaborative Study Group.** 1995. Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. J. Gen. Virol. **76:**2493–2507.
19. **Mizushima, H., M. Hijikata, S. Asabe, M. Hirota, K. Kimura, and K. Shimotohno.** 1994. Two hepatitis C virus glycoprotein E2 products with different C termini. J. Virol. **68:**6215–6222.
20. **Okamoto, H., M. Kojima, M. Sakamoto, H. Iizuka, S. Hadiwandowo, S. Suwignyo, Y. Miyakawa, and M. Mayumi.** 1994. The entire nucleotide sequence and classification of a hepatitis C virus isolate of a novel genotype from an Indonesian patient with chronic liver disease. J. Gen. Virol. **75:**629–635.
21. **Pistello, M., F. Maggi, L. Vatteroni, N. Cecconi, F. Panicucci, G. P. Bresci, L. Gambardella, M. Taddei, A. Bionda, M. Tuoni, and M. Bendinelli.** 1994. Prevalence of hepatitis C virus genotypes in Italy. J. Clin. Microbiol. **32:**232–234.
22. **Pol, S., V. Thiers, J. B. Nousbaum, C. Legendre, P. Berthelot, H. Kreis, and C. Brechot.** 1995. The changing relative prevalence of hepatitis C virus genotypes: evidence in hemodialyzed patients and kidney recipients. Gastroenterology **108:**581–583.
23. **Pozzat, G., M. Moretti, F. Franzin, L. S. Crocé, C. Tiribelli, T. Masayu, S. Kaneko, M. Unoura, and K. Kobayashi.** 1991. Severity of liver disease with different hepatitis C viral clones. Lancet **338:**509.
24. **Shukla, D. D., P. A. Hoyne, and C. W. Ward.** 1995. Evaluation of complete genome sequences and sequences of individual gene products for the classification of hepatitis C viruses. Arch. Virol. **140:**1747–1761.
25. **Simmonds, P., E. C. Holmes, T.-A. Cha, S.-W. Chan, F. McOmish, B. Irvine, E. Beall, P. L. Yap, J. Kolberg, and M. S. Urdea.** 1993. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J. Gen. Virol. **74:**2391–2399.
26. **Tanaka, T., K. Tsukiyama-Kohara, K. Yamaguchi, S. Yagi, S. Tanaka, A. Hasegawa, Y. Ohta, N. Hattori, and M. Kohara.** 1994. Significance of specific antibody assay for genotyping of hepatitis C virus. Hepatology **19:**1347–1353.
27. **Tokita, H., H. Okamoto, H. Iizuka, J. Kishimoto, F. Tsuda, L. A. Lesmana, Y. Miyakawa, and M. Mayumi.** 1996. Hepatitis C virus variants from Jakarta, Indonesia classifiable into novel genotypes in the second (2e and 2f), tenth

(10a) and eleventh (11a) genetic groups. J. Gen. Virol. **77:**293–301.
28. **Tokita, H., H. Okamoto, F. Tsuda, P. Song, S. Nakata, T. Chosa, H. Iizuka, S. Mishiro, Y. Miyakawa, and M. Mayumi.** 1994. Hepatitis C virus variants from Vietnam are classifiable into the seventh, eighth, and ninth major genetic groups. Proc. Natl. Acad. Sci. USA **91:**11022–11026.
29. **Tokita, H., S. M. Shrestha, H. Okamoto, M. Sakamoto, M. Horikita, H. Iizuka, S. Shrestha, Y. Miyakawa, and M. Mayumi.** 1994. Hepatitis C variants from Nepal with novel genotypes and their classification into the third major group. J. Gen. Virol. **75:**931–936.
30. **Valliammai, T., S. P. Thyagarajan, A. J. Zuckerman, and T. J. Harrison.** 1995. Diversity of genotypes of hepatitis C virus in southern India. J. Gen. Virol. **76:**711–716.
31. **Widjaja, S., S. Li, S. Ali, S. Simon, A. Sulaiman, L. A. Lesmana, and S. H. Yap.** 1995. Hepatitis C virus RNA detection and HCV genotype in patients with chronic non-A, non-B hepatitis in Jakarta. J. Virol. Methods **51:**169–175.