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Short Communication

Viral Load in Indonesian Patients with Chronic Liver Disease and in Blood Donors Infected with Different Subtypes of Hepatitis C Virus

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SUMMARY: The viral load of different hepatitis C virus (HCV) subtypes, including the globally distributed HCV-1b and the unique Indonesian subtype HCV-1c, was analyzed using serum samples obtained from Indonesian blood donors and patients with chronic liver disease. The mean viral load of HCV-1c was comparable with that of HCV-1b, suggesting that HCV-1c is as pathogenic as HCV-1b. On the other hand, the mean viral load of HCV-2a was lower than that of HCV-1b or HCV-1c, with this result being consistent with previous observations. Interestingly, some HCV-2a strains were associated with a high viral load that was almost equivalent to that of HCV-1b and HCV-1c. This result implies the possibility that there exists a minor fraction of HCV-2a strains that cause higher levels of viremia compared with the majority of ordinary HCV-2a strains.

Hepatitis C virus (HCV) readily establishes chronic persistent infection that often results in chronic hepatitis (CH) and more deteriorating diseases such as liver cirrhosis (LC) and hepatocellular carcinoma (HCC). HCV is classified into at least six clades (formerly called genotypes), each of which can be further divided into a number of subtypes (1-3). The prevalence of each HCV subtype has been reported to vary in different geographical areas (1, 3-6). In addition, viral pathogenicity and sensitivity to interferon (IFN) treatment appear to vary among the subtypes. We previously reported on the prevalence of anti-HCV antibodies and that of each HCV subtype among various populations, including healthy blood donors, patients with liver disease, and patients on maintenance hemodialysis, in Surabaya, Indonesia (5-7). In the course of the study, we identified a unique Indonesian subtype, HCV-1c (formerly referred to as HCV-1d), which has been found almost exclusively in Indonesia (4-6).

In addition to viral subtypes, the HCV RNA level in the serum is an important factor. It has been reported to correlate well with liver damage. Moreover, lower pretreatment serum HCV RNA levels have been shown to be associated with a better response to IFN therapy (8). Therefore, the determination of HCV viral load and HCV subtypes has a clinical importance. In the present paper, we describe the possible relationship between viral load and HCV subtypes in healthy blood donors and in patients with CH, LC, and HCC in Surabaya, Indonesia.

Sera were obtained from the Red Cross Blood Transfusion Center and Dr. Soetomo Hospital, Faculty of Medicine, Airlangga University, Surabaya, between August 1995 and June 1997. These samples were tested for anti-HCV antibodies by enzyme-linked immunosorbent assay (UBI HCV EIA, United Biologicals, Inc., New York, N.Y., USA; Ortho HCV Ab ELISA Test II, Ortho Diagnostics, Inc., Tokyo), and positive sera samples were further tested for HCV RNA by a reverse transcription-polymerase chain reaction (RT-PCR) that was designed to amplify a portion of the NS5B region of the HCV genome (5, 6). Nucleotide sequences of the amplified NS5B fragments were determined, and based on these sequences a subtype was assigned to each HCV strain, as described previously (5, 6). Levels of HCV viral load were assessed by using a commercially available kit (Amplicor HCV Monitor Test, ver. 1.0; Roche Diagnostic Systems, Inc., Branchburg, N.J., USA) according to the manufacturer's instructions. In addition, serum alanine aminotransferase (ALT) levels were determined by using the Granutest ALAT (Merck, Darmstadt, Germany). Normal ALT levels were <23 U/liter for men and <19 U/liter for women when tested at 25°C.

We analyzed the possible relationship between HCV viral load and age, HCV subtypes, or ALT levels in blood donors. Blood donors under 40 years of age were all infected with HCV-2a, with a mean ALT level of 29.4 U/liter and mean HCV viral load of 3.4 logarithm titer/ml (Table 1). In contrast, nearly half of the blood donors over 40 were infected with HCV-1b or HCV-1c, with a mean ALT level of 40.4 U/liter and mean HCV viral load of 5.6 logarithm titer/ml. The difference in the mean viral load between the two groups was statistically significant ($P< 0.005$). Blood donors over 40 with HCV-2a showed a mean ALT level and viral load practically the same as those of donors under 40. These results suggest the possibility that HCV genotype 1 strains, such as HCV-1b and HCV-1c, have the capacity to cause higher degrees of viremia and, consequently, more severe liver damage, which is indicated by elevated ALT levels. A similar observation, reported previously, was that patients infected with HCV-1a or HCV-1b were more viremic than those infected with HCV-3a (9). Our result also implies a possible shift of the prevalent HCV subtypes among blood donors in Surabaya, from HCV-1b and HCV-1c to HCV-2a. It appears that HCV-2a has been more recently introduced, and is currently prevailing, almost exclusively, among blood donors. Such a changing pattern of HCV subtype prevalence over time has also been reported for patients on maintenance hemodialysis and for kidney

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Table 1. HCV viral load and ALT levels in blood donors infected with HCV-1b, HCV-1c or HCV-2a

Age group (year)	HCV subtype	No. of samples	ALT level (U/liter)	Viral load[1] (range)
<40	2a	6	29.4±19.9	3.4±0.6 (<3.0-4.4)
≥40	1b or 1c	6	40.4±15.8	5.6±0.3[2] (5.3-6.0)
	2a	8	27.7±15.2	3.7±0.7 (<3.0-4.8)

[1] Mean titers (Log_{10}HCV RNA copies/ml) ± SD.
[2] $P<0.005$, compared with age group <40, or age group ≥40, HCV-2a.

recipients (10). If this is the case, the prevalence of HCV-2a among patients with chronic liver disease in Surabaya will increase in the future.

To confirm the possibly higher viral load observed with HCV-1b and HCV-1c, the viral load of each subtype in all patient groups was analyzed. The results obtained clearly demonstrated that the mean viral titers of HCV-1b and HCV-1c were significantly higher than that of HCV-2a (Figure). Table 2 also shows a general tendency of HCV-1b and HCV-1c to be associated with higher viral load than that of HCV-2a in patients with chronic liver disease; the difference observed among groups in CH was statistically significant while the differences observed in LC and HCC were not, probably due to the small numbers of samples tested. It should also be noted that the mean viral load of HCV-1c was practically the same as that of HCV-1b.

While most HCV-2a strains are associated with a low viral load, our present result also implies the possibility that there exists a distinct, though minor, group of HCV-2a strains that are associated with a high viral load (Figure). It is tempting to speculate that this particular group of HCV-2a is more pathogenic, and is more responsible than the majority of ordinary HCV-2a strains with a low viral load for the occurrence of CH, LC, and HCC. Follow-up study of blood donors infected with HCV-2a of high viral titers and those of low titers is necessary to investigate whether this speculation is correct. Also, a sequence comparison between HCV-2a isolates of high titers and those of low titers, as well as a comparison between HCV-2a isolates obtained from long-term healthy carriers and those obtained from patients with LC and HCC, may help us understand the molecular mechanism(s) that underlies the pathogenicity of HCV. Such sequence

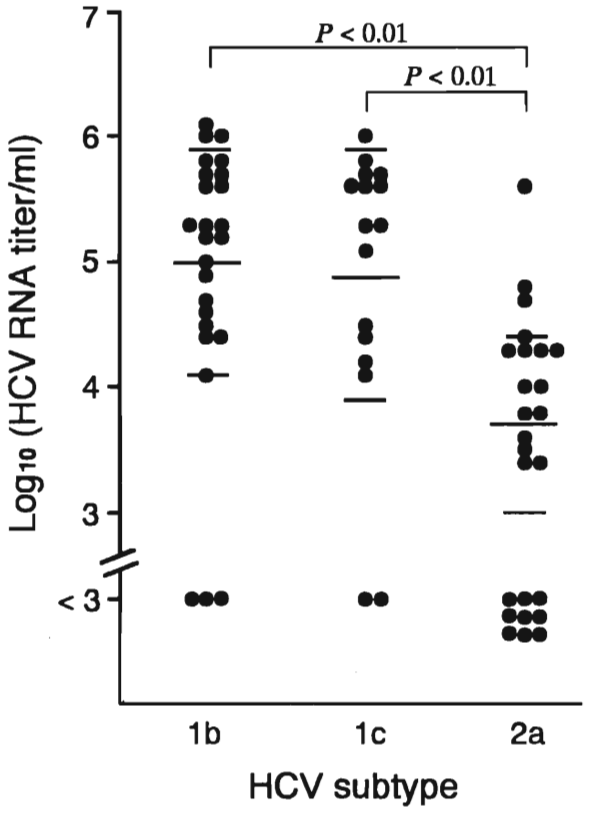

Figure. HCV viral load of different HCV subtypes in sera of blood donors and patients with CH, LC, and HCC. Each dot represents the viral load of a participant in this study. Mean values and standard deviations are shown by long and short horizontal bars, respectively.

Table 2. HCV viral load and ALT levels in blood donors, CH, LC, and HCC infected with different HCV subtypes

Patient group	Subtype	No. of samples	Age (years)	ALT level (U/liter)	Viral load[1] (range)
Blood donor	1b	4	51.7±2.6	31.4±5.6	5.7±0.3 (5.3-6.0)
	1c	2	51.5±4.9	58.4±13.4	5.4±0.2 (5.3-5.6)
	2a	14	44.0±13.8	28.5±16.7	3.5±0.6[2] (<3.0-4.8)
CH	1b	6	62.3±3.1	30.0±14.2	5.1±0.7 (4.4-6.1)
	1c	4	44.3±21.4	29.0±8.4	5.1±0.7 (4.1-5.7)
	2a	3	66.0±3.0	35.7±25.4	3.8±0.4[3] (3.5-4.3)
LC	1b	6	62.0±4.6	51.3±73.2	4.9±1.1 (<3.0-6.0)
	1c	6	62.5±5.7	51.7±31.8	5.1±1.1 (<3.0-6.0)
	2a	5	61.8±4.3	35.4±14.2	4.0±1.1 (<3.0-5.6)
HCC	1b	9	58.3±10.6	46.4±22.3	4.6±1.0 (<3.0-5.8)
	1c	4	58.3±1.3	62.6±32.7	4.3±1.1 (<3.0-5.7)
	2a	3	63.3±5.8	24.3±15.3	3.9±0.5 (3.4-4.3)

[1] Mean titers (Log_{10}HCV RNA copies/ml) ± SD.
[2] $P<0.005$, compared with HCV-1b and HCV-1c.
[3] $P<0.05$, compared with HCV-1b and HCV-1c.

comparisons are currently underway in our laboratory.

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