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Prevalence and Clinical Significance of SEN Virus Infection Among Volunteer Blood Donors in Southern Taiwan

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Of the eight different isolates of SEN virus (SENV), SENV-D and SENV-H have been suggested associated with transfusion-associated hepatitis. The prevalence and clinical significance of these two SENV strains among blood donors in southern Taiwan were investigated in this study. Sera of 223 blood donors who were negative for serum hepatitis B surface antigen (HBsAg) and third-generation HCV antibody (anti-HCV) from a blood center of southern Taiwan were tested for alanine aminotransferase (ALT), GB virus C/hepatitis G virus (GBV-C/HGV) anti-envelope protein 2 (anti-E2) antibody and RNA, and SENV-D and -H DNA. Of the 223 donors, the prevalence of SENV-D and/or -H (SENV-D/H), SENV-D, SENV-H DNA, GBV-C/HGV RNA, and anti-E2 were 24.2, 19.7, 5.8, 2.2, and 8.5%. The donors with SENV-D DNA had a significantly higher mean age than those without (31.2 ± 10.9 vs. 27.5 ± 8.3 years; $P = 0.014$). No association between positive SENV DNA and gender, GBV-C/HGV exposure, mean ALT level, or abnormal ALT was found. Based on multiple logistic regression analysis, the increased age was the only independent factor associated with positive SENV-D DNA (odds ratio, 1.042; 95% confidence interval, 1.01–1.08). Nearly a fourth of blood donors in southern Taiwan were infected by SENV-D/H, with SENV-D more prevalent than SENV-H. Patients with higher ages have a higher prevalence of SENV-D. SENV-D or SENV-H infection was not associated with ALT levels.

KEY WORDS: alanine aminotransferase; blood donor; GBV-C/HGV; SENV.

Taiwan is a hepatitis B endemic area (1). The prevalence rate of chronic hepatitis C (CHC), although ranging from 0.95 to 2.6% in the general population (1, 2), has been reported to be up to 57.9% in some communities in southern Taiwan (3). Chronic hepatitis B virus (HBV) and chronic hepatitis C virus (HCV) infections account for the majority of cases with abnormal liver biochemistry in Taiwan. Screening tests have been introduced into the blood banks routinely for HBV and HCV to avoid the transmission of hepatitis. Nevertheless, sporadic cases of posttransfusion hepatitis are still observed occasionally. More efforts have been devoted to identify potential causative viral agents responsible for these patients.

A flavivirus-like virus named GB virus C (GBV-C) and another isolate of GBV-C, hepatitis G virus (GBV-C/HGV), have been claimed to be associated with different types of hepatitis (4–6). However, other and our previous reports have indicated that GBV-C/HGV does not account for liver diseases (7–9). A novel nonenveloped, single-stranded DNA virus was found in 1997 and designated TT virus (TTV) after the initials (T.T.) of the index patient (10, 11). TTV, with a circular genome that comprises 3852 bases with a particle size of 30–50 nm, resembles the

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circoviridae (12). There were some reports suggesting that TTV may be associated with elevated ALT levels (10, 11, 13, 14) and may play a role in the development of chronic liver diseases of unknown etiology (10, 15). In contrast, some investigations revealed that the presence of TTV DNA was not correlated with abnormal ALT levels (16, 17) or liver injury (18, 19). Therefore, the pathogenicity and hepatopathic effects of GBV-C/HGV and TTV infection remain controversial.

A new family of DNA viruses was recently isolated and designated SEN virus (SENV), after the initials of the infected patient (20, 21). SENV is a small, single-stranded circular DNA virus that is distantly related to the large TT virus family and eight different variants (A–H) have been shown by the phylogenetic analysis of SENV (22). Two strains of SENV (SENV-D and SENV-H; SENV-D/H) are more prevalent in patients with transfusion-associated non-AE hepatitis than in healthy blood donors, which suggested significant associations between SENV-D and SENV-H and transfusion-associated hepatitis (20, 23). However, the association of SENV infection with hepatocyte damage or the aminotransferase level remained uncertain. In the present study, we investigated the prevalence and clinical significance of SENV-D and SENV-H infection among blood donors from southern Taiwan.

METHODS

Subjects. Two hundred twenty-three volunteer blood donors from a blood center in Kaohsiung, the largest city in southern Taiwan, were enrolled in the study. There were 167 men and 56 women ranging in age from 18 to 64 years (mean ± standard deviation [SD]: 28.2 ± 8.9 years). All the serum samples were negative for both HBsAg and anti-HCV. The studies are in accordance with the Helsinki Declaration of 1995 and were approved by the Ethical Committee of Kaohsiung Medical University Hospital.

Laboratory Tests. Third-generation HCV anitbody and HBsAg were detected with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Abbott, North Chicago, IL). Alanine aminotransferase (ALT) (normal upper limit of serum ALT = 34 IU/liter) was measured on a multichannel autoanalyzer.

Detection of SENV-D and SENV-H by Polymerase Chain Reaction (PCR). The presence of SENV-D and SENV-H DNA was determined by PCR using primers as described previously with modification (22). Briefly, total DNA was extracted from 200 μL of serum with the QIAamp blood kit (QIAGEN Ltd., Hilden, Germany) and resuspended in 50 μL of elution buffer. For the PCR, 25 μL of reaction mixture containing 2.5 μL of the DNA sample, 1× PCR buffer (10 m*M* Tris–HCl [pH 9.0] , 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% gelatin, and 0.1% Triton X-100), a 200 μM concentration of each dNTP, 100 ng of each primer (sense primer for SENV-D, 5′-GTAACTTTGCGGTCAACTGCC-3′; sense primer for SENV-H, 5′-GGTGCCCCTWGTYAGTTGGCGGTT-3′ [W = A or T]; universal antisense primer, 5′-CCTCGGTTKSAAAK GTYTGATAGT-3′ [K = G or T, S = C or G, and Y = C or T]), and 1.25 U of Taq DNA polymerase was amplified in a thermal cycler (Perkin–Elmer Cetus) for 35 cycles consisting of denaturation at 95°C for 45 sec, primer annealing for 45 sec at 58°C for SENV-D and at 62°C for SENV-H, and extenstion at 72°C for 45 sec, with a final extension step at 72°C for 7 min. The amplified products (231 bp for SENV-D and 230 bp for SENV-H) were separated in 3% agarose gel electrophoresis and stained with ethidium bromide. Precautions against amplicon contamination were taken during the procedures.

Detection of GBV-C/HGV RNA and Anti-E2 Antibody in Serum. GBV-C/HGV RNA was detected by nested neverse transcription PCR (RT-PCR) using primers targeting the 5' UTR as described previously (24). The anti-E2 antibody was measured by an enzyme-linked immunosorbent assay from Boehringer Mannheim (GmbH, Germany), strictly according to be manufacturer's instructions (25).

Statistical Analyses. Descriptive statistics such as means and proportions were calculated: Frequency was compared between groups using the chi-square test or Fisher's exact test, and group means were compared using Student's *t* test. Stepwise logistic regression method was used to analyze the study data. Odds ratios (ORs) and their associated 95% confidence intervals (CIs) were used to quantify the magnitude of their associations. $P < 0.05$ was considered statistically significant.

RESULTS

Prevalence of SENV-D/H DNA. Of 223 blood donors without HBsAg and anti-HCV, the PCR results showed that 44 (19.7%) were positive for SENV-D and 13 (5.8%) were positive for SENV-H. With three (1.4%) patients positive for both SENV-D and SENV-H DNA (SENV-D/H co-infection), the overall prevalence of SENV-D/H was 24.2%.

GBV-C/HGV Viremia and Anti–E2 Antibodies. GBV-C/HGV RNA was present in five (2.2%) of the studied samples. With 19 (8.5%) positive for anti-E2 antibodies and 2 (0.4%) positive for both serum GBV-C/HGV RNA and anti-E2 antibodies, the prevalence of HGV exposure, defined as positive for serum GBV-C/HGV RNA and/or anti-E2, was 9.9%. There was no association between the presence of GBV-C/HGV RNA and SENV-D/H or between the GBV-C/HGV exposure rate and SENV-D/H (Table 1).

Serum ALT Levels and SENV Viremia. The mean serum ALT level of the studied samples was 14.5 ± 9.8 IU/L. Nine donors had elevated ALT levels (range: 37–73 IU/L). The mean ALT level was similar in SENV-D/H DNA-positive and -negative donors (15.0 ± 10.0 vs. 14.4 ± 9.8 IU/L) and also similar in SENV-D and SENV-H DNA-positive and -negative donors (16.0 ± 10.4 vs. 14.2 ± 9.7 and 11.2 ± 6.7 vs. 14.8 ± 10.0 IU/L, respectively). Also, no association between mean ALT levels and GBV-C/HGV viremia or exposure (10.8 ± 8.4 vs. 14.6 ± 9.9 and 15.9 ± 15.2 vs. 14.4 ± 9.1 IU/L, respectively) was

TABLE 1. COMPARISON OF CLINICAL CHARACTERITICS BETWEEN INDIVIDUALS WITH AND INDIVIDUALS WITHOUT SENV DNA AMONG BLOOD DONORS FROM SOUTHERN TAIWAN

	D and/or H		*D*		*H*	
	Positive (N = 54)	*Negative (N = 169)*	*Positive (N = 44)*	*Negative (N = 179)*	*Positive (N = 13)*	*Negative (N = 210)*
Gender (male %)	39 (72.2)	128 (75.7)	31 (70.5)	136 (76.0)	11 (84.6)	156 (74.3)
Age (year)	30.2 ± 10.4^a	27.6 ± 8.3^a	31.2 ± 10.9^b	27.5 ± 8.3^b	27.0 ± 8.3	28.3 ± 8.9
ALT (IU/L)	15.0 ± 10.0	14.4 ± 9.8	16.0 ± 10.4	14.2 ± 9.7	11.2 ± 6.7	14.8 ± 10.0
GBV-C/HGV RNA (positive, %)	1 (1.9)	4 (2.4)	1 (2.3)	4 (2.2)	0 (0)	5 (2.4)
GBV-C/HGV exposure (positive, %)	7 (13.0)	15 (8.9)	5 (11.4)	17 (9.5)	3 (23.1)	19 (9.1)
Blood donation (first time, %)	11 (20.4)	39 (23.1)	10 (22.7)	40 (22.4)	1 (7.7)	49 (23.3)

Note. Values are given as mean ± SD. [a] $P = 0.054$. [b] $P = 0.014$. ALT, alanine aminotransferase, GBV-C/HGV, GB virus C/hepatitis G virus.

observed (Table 1). Of the nine donors with elevated ALT levels, three were SENV-D viremic and all were negative for SENV-H and GBV-C/HGV RNA. The prevalence of SENV infection between normal and abnormal ALT levels was similar (23.8 vs. 33.3%; $P = 0.2$).

Clinical Features of SENV-D/H Infection. The comparison of clinical characteristics between SENV viremic and nonviremic blood donors is shown in Table 1. The mean age was higher in SENV-D/H DNA-positive (30.2 ± 10.4 years) blood donors than -negative ones (27.6 ± 8.3 years) ($P = 0.054$). The significantly higher mean age among subjects with positive SENV-D DNA than among those without SENV-D DNA was noted (31.2 ± 10.9 vs. 27.5 ± 8.3 years; $P = 0.014$). Nevertheless, the mean age was similar in SENV-H DNA-positive and -negative donors (27.0 ± 8.6 vs. 28.3 ± 8.9 years). No association between positive SENV DNA and gender or the prevalence of GBV-C/HGV exposure was found. Based on multiple logistic regression analysis, increased age was identified to be the only independent factor associated with positive SENV-D DNA (OR and 95% CI, 1.042 and 1.01–1.08).

Times of Blood Donation and SENV Viremia. Fifty of the 223 blood donors (22.4%) donated blood for the first time. When the SENV DNA-positive rates between individuals with first and those with repeated donations were compared, no significant difference was observed (SENV-D/H, 22.0 vs. 24.9%; SENV-D, 20.0 vs. 19.7%; SENV-H, 2.0 vs. 6.9%).

DISCUSSION

According to previous studies, the SENV-D and -H strains, of the eight different strains (A–H), were more prevalent in patients with transfusion-associated non-AE hepatitis than in healthy blood donors, which suggested their significant associations with transfusion-associated hepatitis (20, 23). The prevalence of SENV-D or -H among blood donors was reported to be 1.8% (23) in the United States and 10 to 22% in Japan (26, 27). In the present study, the prevalence of SENV-D/H infection in healthy blood donors from southern Taiwan (24.2%) seemed similar to the finding from Japan (27) and higher than the reports of 15% from northern Taiwan by Kao and coworkers (28). It is noteworthy that a different prevalence of these two SENV isolates, D and H, between southern and northern Taiwan exists. Kao *et al*. reported that the prevalence of SENV-H was two to seven times higher than that of SENV-D in different northern Taiwanese subjects including healthy adults, patients with acute or chronic hepatitis, intravenous drug users, hemophiliacs, thalassemic patients, and patients on maintenance hemodialysis (28, 29). In their studies the prevalence of SENV-D and -H among healthy adults was 2 and 15%, respectively. We demonstrated in our study that the prevalence of SENV-D was higher than that of SENV-H among southern Taiwan blood donors (19.7 and 5.8%), which is similar to the findings of most other studies around the world showing that SENV-D is more prevalent than SENV-H (27, 30, 31). The results indicated a different geographic distribution of SENV variants in Taiwan. As observed in previous studies, differences in prevalence and genotype distribution of HCV were also noted between southern and northern Taiwan (32–34). We supposed that the independent SENV-D/H outbreaks take place across southern and northern Taiwan.

There was a higher mean age among donors who were SENV-D viremic than those who were nonviremic. However, no significant correlation between age and prevalence of SENV-H observed. The cause of discrepancy between trends of change in prevalence of SENV-D and -H was not clear. The possible explanations to clarify the issues such as different exposure rates or routes of infection as well as different rates of spontaneous clearance between these two strains make further large-scale and longitudinal studies necessary.

The clinical implications and etiological importance in association with liver diseases of SENV infection still

remain undetermined. Being more prevalent in serum of these patients and less frequent in serum samples from healthy blood donors, SENV-D and SENV-H were considered to be associated with transfusion-associated non-A–E hepatitis (20, 21). However, several reports did not support that SENV-D/H was the causal agent for non-A–E fulminant hepatitis (26, 28). In the present study, we failed to show an association between infection with SENV-D/H, SENV-D, or SENV-H and abnormal ALT levels. Besides, SENV viremia was not correlated with ALT levels. Our data, comparable with other reports (26, 28), denied a role for SENV-D/H as causal agents in the development of hepatitis. Likewise, GBV-C/HGV exposure was demonstrated to be irrelevant to ALT levels as in previous reports (7–9). Furthermore, we showed repeated donations did not decrease the SENV viremia rate in blood donors. This depicts the fact that available screening tests for donated blood nowadays cannot prevent SENV infection entirely. On the other hand, because the majority of SENV-infected donors have normal ALT levels, there is no impact on SENV transmission even if donors with elevated ALT levels are prevented from giving further blood. Since the possible correlation between SENV infection and liver diseases has not been determined, it is not, indeed, suggested nowadays to screen donated blood for SENV from a cost–benefit point of view.

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