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The prevalence and clinical characteristics of coinfection of SENV-H among Taiwanese chronic hepatitis C patients with combination therapy of high-dose interferon-alfa and ribavirin

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Abstract

The clinical significance of coinfection of SENV-H among patients with chronic hepatitis C (CHC) and the response to combination therapy with high-dose interferon-alpha (IFN) plus ribavirin in Taiwan are uncertain. A total of 151 (120 histologically proved) naïve CHC patients who received 6 MU IFN thrice a week plus ribavirin for 24 weeks therapy were enrolled in this study. SENV-H DNA was tested by PCR method. Of 151 patients, 29 (19.2%) were positive for SENV-H DNA. The positive SENV-H DNA was significantly associated with HCV genotype 1b than non-1b infection (69.0% versus 43.4%; $P = 0.011$). No other clinical, histopathological and virological factor was related to positive SENV-H DNA. After combination therapy, the rate of sustained viral response (SVR) of HCV and SENV-H were 66.9 and 78.3%, respectively. By multivariate analyses, the significant factors associated with HCV SVR after combination therapy were HCV genotype non-1b, pretreatment HCV RNA levels less than 200,000 IU/mL, and younger age. We conclude that coexistent SENV-H infection, apparently associated with HCV genotype 1b, is found among 19.2% of Taiwanese CHC patients. Both HCV and SENV-H are highly susceptible to combination therapy with high dose IFN and ribavirin and SENV-H coinfection does not affect the HCV response.

Keywords: Combination therapy; HCV; Interferon; Ribavirin; SENV-H

1. Introduction

Hepatitis C virus (HCV) is the major etiologic agent in parenterally transmitted non-A non-B hepatitis and leads to chronic liver disease and primary hepatocellular carcinoma (Alter et al., 1992; Lauer and Walker, 2001). Being a hepatitis B endemic area (Chuang et al., 1993), the prevalence rate of chronic hepatitis C (CHC), although ranging from 0.95 to 2.6% in the general population (Chen et al., 1991; Chuang et al., 1993), has been reported to be up to 57.9% in some communities in southern Taiwan (Wang et al., 1999). The combination therapy with interferon-alfa (IFN) and ribavirin has been considered as first-line therapy for CHC. Previous reports have demonstrated the increased rate of sustained viral response (SVR) to 31–43% after combination therapy for 24 or 48 weeks than 6–19% of IFN monotherapy (McHutchison et al., 1998; Poynard et al., 1998). With a high-dose IFN monotherapy in our previous study, the rate of HCV sustained viral response (SVR) achieved 41.2% (Dai et al., 2003) that was similar to the report of 43% by Lai et al. (1996) in Taiwan after combination therapy with standard IFN dose. The benefits of high-dose IFN in the combination therapy for CHC may exist.

Although the flavivirus-like virus named GB virus C/hepatitis G virus (GBV-C/HGV) and the non-enveloped, single-stranded DNA virus designed TT virus (TTV) have been claimed to be associated with chronic non-AE hepatitis (Simons et al., 1995; Nishizawa et al., 1997), most studies have indicated that neither virus is associated with liver disease (Alter et al., 1997; Yu et al., 2001; Kao et al., 2000; Dai et al., 2002a,b). A new family of DNA viruses was recently isolated and designated as SEN virus (SENV), after the initials of the infected patient (Mushahwar, 2000; Bowden, 2001). SENV is a single-stranded circular DNA virus, a member of the *Circoviridae*, that is distantly related

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to the large TT virus family with eight different variants (A–H) shown by the phylogenetic analysis (Tanaka et al., 2001). Two strains of SENV (SENV-D and SENV-H) are more prevalent in patients with transfusion-associated non-AE hepatitis than in healthy blood donors that suggested the significant associations between SENV-D and SENV-H and transfusion-associated hepatitis (Mushahwar, 2000; Umemura et al., 2001a). However, the association between SENV infection and liver cell damage and the clinical significance of SENV infection in combination with HCV infection remains undefined. For patients with CHC, the report from Rigas et al. (2001) suggested that coinfection with SENV might affect adversely the outcome of treatment with combination therapy. Nevertheless, the study by Kao et al. (2003) did not support their findings.

The objectives of the present study were to elucidate the prevalence and clinical implications of coinfection of SENV-H on biochemical, pathological and virological profile among patients with CHC and, on the other hand, to determine the response of HCV and SENV-H to combination therapy with high-dose IFN and ribavirin. Furthermore, the predictive factors for HCV SVR and the influence of concurrent SENV-H infection on HCV response were investigated.

2. Materials and methods

2.1. Patients

Between June 1998 and March 2001, a total of 151 Taiwanese naïve chronic hepatitis C patients in the clinics of hepatological division of the Kaohsiung Medical University Hospital, 88 men and 63 women, aged between 20 and 72 years (mean 47.9 ±11.4 years) were enrolled in the study. All patients were diagnosed as chronic HCV infection based on continuous positive for second-generation antibody to HCV (anti-HCV) in serum for more than 6 months and positive for HCV RNA. Liver Biopsies were undergone in 120 patients and the disease activity grade and fibrosis stage were quantitatively scored according to the histological activity index scoring system described by Knodell et al. (1981), Scheuer (1991) and Desmet et al. (1994). Patients who were positive for hepatitis B surface antigen (HBsAg), had human immunodeficiency virus type I infection, autoimmune liver disease, metabolic liver diseases including α-1 anti-trypsin deficiency, hemochromatosis or Wilson's disease, alcoholic liver disease or intravenous drug abuse were excluded. Eighteen (1.2%) patients were diagnosed as liver cirrhosis histologically or, in the absence of a liver biopsy, by a compatible ultrasonographic and clinical picture. All the serum samples, when collected from patients at the time of their evaluation, were stored at −70 °C before testing. The study had been approved by the ethics committee of Kaohsiung Medical University Hospital and all patients had given their informed consents.

2.2. Laboratory tests

Serum HBsAg was assayed using commercially available kits (General Biological HBsAg radio-immunoassary (RIA); General Biological Cooperation, Taiwan) and second-generation HCV antibody (anti-HCV) was 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/L) was measured on a multichannel autoanalyzer.

2.3. Detection of SENV-H DNA

The presence of SENV-H DNA was determined by PCR by using primers as described previously with modification (Tanaka et al., 2001). 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 mM Tris–HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, and 0.1% Triton X-100), 200 μM of each deoxynucleoside triphosphate, 100 ng of each primer (sense primer for SENV-H, 5′-GGTGCCCCTWGTYAGTTGGCGGTT-3′ (W = A or T); antisense primer, 5′-CCTCGGTTKSAAAKGTYTGATAGT-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 s, primer annealing for 45 s at 62 °C, and extension at 72 °C for 45 s, with a final extension step at 72 °C for 7 min. The amplified products (230 bp) were separated in 3% agarose gel electrophoresis and stained with ethidium bromide.

2.4. Detection/quantification of serum HCV RNA and genotyping

Detection of serum HCV RNA was performed using a standardized automated qualitative RT-PCR assay (COBAS AMPLICOR Hepatitis C Virus Test, Version 2.0; Roche, Branchburg, NJ, USA). The detection limit was 50 IU/mL. HCV genotypes 1a, 1b, 2a, 2b and 3a were determined by amplification of the core region using genotype-specific primers described by Okamoto et al. (1993). Pretreatment HCV RNA levels were determined by using the branched DNA assay (Quantiplex HCV RNA 3.0, Bayer, Emeryville, CA), performed strictly in accordance with the manufacturer's instructions. The quantification limit was 615 IU of HCV RNA per mL.

2.5. Combination therapy with high-dose IFN and ribavirin

All 151 patients received combination therapy for 24 weeks using IFN subcutaneously at a dosage of 6 million units (MU) three times weekly and ribavirin by mouth at a

dosage of 1000 (if body weight was <75 kg) or 1200 mg (if body weight was ≥75 kg) daily in three divided doses. After the cessation of therapy, they all received 24 weeks of follow up for evaluating the response. The presence of HCV RNA in the serum was determined at week 24 and 48. A SVR for HCV was defined as patients who had negative HCV RNA by RT-PCR assay 24 weeks after the cessation of combination therapy. All other patients were defined as non-responders (NR). To evaluate the response of SENV-H, the presence of SENV-H DNA was determined at week 24 and 48. An end-of-treatment virologic response (ETVR) and a SVR for SENV-H were indicated by negative PCR results at week 24 and 48, respectively.

2.6. Statistical analyses

Descriptive statistics such as means and proportions were calculated: Frequency was compared between groups using the χ^2-test or Fisher's exact test, and group means were compared using the *t*-test. The presence of a statistical significance was inferred when *P* was less than 0.05. Serum HCV RNA levels were expressed as the mean ± standard deviation after logarithmic transformation of original values. 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.

3. Results

3.1. Study population

Of all 151 naïve CHC patients receiving combination therapy, 18 (11.9%) had liver cirrhosis. The HCV genotype distribution was as follows: 1b in 73 (48.3%) patients, 2a in 46 (30.5%) patients, 2b in 16 (10.6%) patients, mixed in 12 (7.9%) patients and unclassified in 4 (2.7%) patients. The mean pretreatment ALT and HCV RNA levels was 136.9 ± 168.9 IU/L and 5.79 ± 0.67 log IU/mL, respectively, with 131 (86.8%) and 95 (62.9%) patients having abnormal ALT levels and high serum HCV levels (>200,000 IU/mL). Of 120 patients undergoing liver biopsies, the mean scores for peri-portal necrosis, intralobular necrosis, portal inflammation (grading) and fibrosis were 1.11 ± 1.31, 0.53 ± 0.90, 1.95 ± 1.23, 3.56 ± 2.48 and 1.25 ± 1.34, respectively.

3.2. SENV-H viremia in chronic hepatitis C patients

Of 151 CHC patients with combination therapy in the present study, 29 patients were positive for SENV-H DNA showing a prevalence of 19.2%. The comparison of clinical characteristics between patients with and without SENV-H coinfection was shown in Table 1. The positive rate of SENV-H DNA was significantly higher among patients with HCV genotype 1b infection than those who harbored genotype non-1b (20/73, 23.4% versus 9/78, 11.5%; $P = 0.011$). No other clinical and virological factor was

Table 1
Comparison of clinical characteristics between individuals with and without SEN virus-H viremia in chronic hepatitis C patients

	No.	SENV-H DNA no. (%)		*P*
		Positive	Negative	
Patient no.	151	29	122	
Male sex (%)	88	17 (58.6)	71 (58.2)	NS
Age (year)	151	47.4 ± 14.4	48.1 ± 10.7	NS
Serum ALT (IU/L)		120.3 ± 104.3	140.8 ± 181.0	NS
Normal (≤34 IU/L)	20	3 (10.3)	17 (13.9)	NS
Abnormal (>34 IU/L)	131	26 (89.7)	105 (86.1)	
HCV RNA levels (log IU/mL)	151	5.72 ± 0.72	5.81 ± 0.66	NS
High level (≥200,000 IU/mL)	95	17 (58.6)	78 (63.9)	NS
Low level (<200,000 IU/mL)	56	12 (41.4)	44 (36.1)	
HCV genotype				0.011
1b (%)	73	20 (23.4)	53 (76.6)	
Non-1b (%)	78	9 (11.5)	69 (88.5)	
Cirrhosis (%)	18	2 (7.1)	16 (13.1)	NS
Histology (HAI scores)	120	19	101	
Peri-portal necrosis		1.16 ± 1.26	1.10 ± 1.33	NS
Intralobular necrosis		0.42 ± 0.77	0.55 ± 0.92	NS
Portal inflammation		1.89 ± 1.37	1.96 ± 1.27	NS
Total score (grading)		3.47 ± 2.12	3.57 ± 2.55	NS
Fibrosis	120	1.26 ± 1.28	1.25 ± 1.35	NS

Note: results are expressed as mean ± S.D. unless otherwise noted.

Table 2
Comparison of clinical and virological features between sustained virologic responders (SVR) and non-responders (NR) of chronic hepatitis C patients after combination therapy

	No.	HCV response no. (%)		*P*
		SVR ($n = 101$)	NR ($n = 50$)	
Male sex (%)	88	58 (65.9)	30 (34.1)	NS
Age (year)	151	46.3 ± 11.1	51.4 ± 11.4	0.009
Serum ALT (IU/L)		143.3 ± 193.0	123.9 ± 105.3	NS
Normal (≤34 IU/L)	20	16 (15.8)	4 (8.0)	NS
Abnormal (>34 IU/L)	131	85 (84.2)	46 (92.0)	
HCV RNA levels (log IU/mL)	151	5.74 ± 0.70	5.89 ± 0.62	NS
High level (≥200,000 IU/mL)	95	57 (56.4)	38 (76.0)	0.019
Low level (<200,000 IU/mL)	56	44 (43.6)	12 (24.0)	
HCV genotype 1b	73	29 (39.7)	44 (60.3)	<0.0001
Non-1b	78	72 (92.3)	6 (7.7)	
Cirrhosis (%)	18	9 (8.9)	9 (18.4)	NS
Positive SENV-H DNA (%)	29	16 (55.2)	13 (44.8)	NS
Histology (HAI scores) no.	120	84	36	
Peri-portal necrosis		1.11 ± 1.33	1.11 ± 1.30	NS
Intralobular necrosis		0.64 ± 0.98	0.28 ± 0.61	0.040
Portal inflammation		1.90 ± 1.26	2.06 ± 1.17	NS
Total score (grading)		3.63 ± 2.59	3.39 ± 2.22	NS
Fibrosis	120	1.17 ± 1.32	1.44 ± 1.38	NS

Note: results are expressed as mean ± S.D. unless otherwise noted.

related to positive SENV-H DNA. Among 120 patients undergoing liver biopsies, all of mean scores were similar between SENV-H DNA-positive and -negative CHC patients (Table 1).

3.3. HCV virologic response to combination therapy

After combination therapy with high dose IFN and ribavirin for 24 weeks, 101 (66.9%) of 151 patients achieved HCV SVR. The clinical and virological features between CHC patients with HCV SVR and NR were shown in Table 2. In comparison between these two groups by univariate analysis, the higher rate of HCV SVR was significantly related to younger ages ($P = 0.009$), lower pretreatment levels of HCV RNA (less than 200,000 IU/mL) ($P = 0.019$), HCV genotype non-1b ($P < 0.001$) and higher HAI score for intralobular regeneration and focal necrosis ($P = 0.04$). No significant association between other clinical and virological factors and HCV response of combination therapy was observed. Based on multivariate regression analyses, the significant factors associated with HCV SVR after combination therapy were HCV genotype non-1b, pretreatment HCV RNA levels less than 200,000 IU/mL, and younger age with the odds ratio and 95% confidence interval of these factors summarized in Table 3.

3.4. Clearance of SENV-H DNA after combination therapy

SENV-H DNA was followed in 23 chronic hepatitis C patients (13 men and 10 women, aged between 20 and 65 years (mean 40.3 ± 13.5 years) concomitant with SENV-H viremia before combination therapy. Their mean ALT level was 129.4 ± 108.1 IU/L (range: 16–467) and 22 patients were abnormal. The HCV genotype distribution was as follows: 1b in 15 patients, 2a in 6 patients, 2b in 1 patient and mixed in 1 patient. One patient was diagnosed as LC. The clinical characteristics and virological features between individuals with and without SENV-H DNA SVR after combination therapy were analyzed and shown in Table 4. Fourteen (60.9%) of the 23 SENV-H DNA-positive patients achieved HCV SVR after combination therapy. At the end of treatment, SENV-H DNA was negative in 14 patients (60.9%).

Table 3
Stepwise logistic regression analysis of factors significantly associated with HCV sustained virologic response (SVR) after combination therapy

Dependent variable	Independent variable	Comparison	Odds ratio (95% CI*)
SEN virus-H viremia	HCV genotypes	1b vs. non-1b	3.06 (1.27–7.37)
HCV SVR	HCV genotypes	1b vs. non-1b	0.06 (0.02–0.19)
	HCV RNA level	High (≥200,000 IU/mL) vs. low (<200,000 IU/mL) level	0.33 (0.11–0.98)
	Age	Per year increased	0.94 (0.90–0.99)

* Confidence interval.

Table 4
Comparison of clinical characteristics and virological features between chronic hepatitis C patients with and without sustained clearance of SEN virus-H after combination therapy

	SENV-H response no. (%)		P
	SVR ($n = 18$)	NR ($n = 5$)	
Male sex (%)	10 (55.6)	3 (60.0)	NS
Age (year)	48.9 ± 13.4	36.8 ± 9.4	NS
Serum ALT (IU/L)	122.8 ± 118.3	153.0 ± 62.2	NS
High HCV RNA level (≥200,000 IU/mL) (%)	13 (72.2)	1 (20.0)	0.056
HCV genotype 1b (%)	12 (66.7)	3 (60.0)	NS
SENV-H ETVR[a] (%)	13 (72.2)	1 (20.0)	0.056
HCV SVR (%)	10 (55.6)	4 (80.0)	NS

Note: results are expressed as mean ± S.D. unless otherwise noted.
[a] End-of-treatment virologic response.

When SENV-H DNA was followed 24 weeks after the cessation of therapy, one of 14 patients (7.1%) had reappearance of serum SENV-H DNA and she was HCV SVR with normal ALT level. Of these 13 patients, despite negative SENV-H DNA at the end of treatment, six cases were HCV NR and their ALT levels were abnormal. Five of nine patients (55.6%) with positive SENV-H DNA at the end of treatment had clearance of SENV-H DNA 24 weeks after the cessation of therapy. Two of them were HCV NR with abnormal ALT levels despite SENV-H SVR achieved. The rate of SVR of SENV-H DNA after combination therapy was 78.3% (18/23). As shown in Table 4, the SVR of SENV-H was higher among patients with ETVR than those who were SENV-H viremia at the end of treatment (72.2% versus 20.0%) and among patients with high HCV RNA level (72.2% versus 20.0%) although the statistical significance were borderline. No other clinical and virological factor was related to SVR for SENV-H.

4. Discussion

The geographic distribution of different SENV variants has been reported. The studies in Japan have demonstrated that SENV-D is more prevalent than SENV-H (Kobayashi et al., 2003; Umemura et al., 2001b, 2002; Shibata et al., 2001). Nevertheless the SENV-H has been reported the predominant strain in the United States (Umemura et al., 2001a). It is interesting that in Taiwan there is also different prevalence of SENV D and H isolates between southern and northern part. Kao et al. (2002, 2003) reported in different northern Taiwanese individuals that the prevalence of SENV-H was two to seven times higher than that of SENV-D. The findings of the present study revealed that 19.2% of Taiwanese patients with chronic hepatitis C were coinfected with SENV-H that was lower than reports of 41% from Kao et al. (2003) and 30% from Germany (Schröter et al., 2002). As we found in our previous studies that the prevalence of SENV-D was higher than that of SENV-H among southern Taiwan blood donors (19.7 and 5.8%) and among patients on maintenance hemodialysis (46.5 and 27.3%) (unpublished data), we demonstrated a marked difference of genotypic distribution of SENV between southern and northern Taiwan.

The clinical significance of SENV infection in combination with HCV infection remains unclear (Rigas et al., 2001; Schröter et al., 2002; Kao et al., 2003). Our data showed that HCV genotype 1b was more prevalent among CHC patients with SENV-H coinfection than among those without SENV-H coinfection. This result was different from that from Kao et al. (2003) showing the relevant between HCV genotype 2a and SENV coinfection and the discrepancy needs further studies. In the present study, the pretreatment mean ALT levels and HCV RNA levels, and the histological scores between CHC patients with and without SENV-H coinfection were compatible. Our data failed to show any influence of SENV-H coinfection on the biochemical and histological characteristics of CHC patients that indicated the irrelevance between severity of liver disease and SENV-H coinfection. The clinical significance of SENV-H is unknown and further studies are necessary.

In the present study, the HCV SVR rate of combination therapy with 6MU IFN and ribavirin for 24 weeks achieved 66.9%. Since the combination therapy with IFN and ribavirin has become the standard of therapy for naïve CHC patients, previous reports have demonstrated the SVR rate of 31–43% after combination therapy for 24 or 48 weeks (McHutchison et al., 1998; Poynard et al., 1998). As previous reports from researchers in Taiwan, the SVR rate of CHC patients was high (40–43%) after combination therapy with IFN 3 MU and ribavirin for 24 weeks (Lai et al., 1996; Kao et al., 2003). Even though the pegylated IFN is now preferred for combination therapy for CHC, in the present study, 66.9% of patients achieved SVR after combination therapy with 6MU IFN and ribavirin for 24 week that may further indicate the favorable results of combination therapy for Taiwanese CHC patients. The positive predictors of HCV SVR to combination therapy with high-dose IFN were elucidated as HCV genotype non-1b, lower pretreatment HCV RNA levels and younger age. Factors including

HCV genotype, pretreatment serum HCV RNA level, ages, fibrosis stage and sex have been reported as predictors of HCV SVR to standard dose combination therapy (Poynard et al., 1998). Whether the increased dose of IFN in combination therapy will overcome the disadvantage of the male sex and sever fibrosis on HCV SVR needs further studies. Previous reports from Rigas et al. (2001) demonstrated that HCV with SENV coinfection affected adversely HCV response to combination therapy with interferon plus ribavirin but the other report denied the relevance between HCV response and SENV coinfection (Kao et al., 2003). Our data here indicates that SENV-H coinfection does not affect the HCV response in the combination therapy with high dose IFN and ribavirin. In other words, it is not necessary and suggested to determine the SENV-H coinfection before CHC patient receiving combination therapy.

After combination therapy with 6MU IFN-alpha and ribavirin for 24 weeks for 23 CHC patients concomitant with SENV-H viremia, the rates of SENV-H viral clearance at the end of follow-up achieved 78.3%. In the previous studies on the response of SENV-H among CHC patients, the sustained response rate of SEN-H have recently reported by Umemura et al. (2002) as 33.3% after high dose interferon monotherapy and by Kao et al. (2003) as 26.8% after combination therapy with 3MU IFN-alpha and ribavirin for 24 weeks. The present study showed the marked increased rate of SENV-H clearance with high dose IFN plus ribavirin therapy that indicated SENV-H was very sensitive to combination therapy, especially when high dose IFN was used. As regards the response of HCV, the rates of HCV SVR in our study (66.9%) with the high dose IFN in the combination therapy was higher than reports from high dose IFN monotherapy (27%) (Umemura et al., 2002) and than combination therapy with 3MU IFN-alpha and ribavirin (40–43%) (Lai et al., 1996; Kao et al., 2003). The additional benefits gained by the increased IFN dose in combination therapy seem significant in eradication of not only SENV-H but also HCV. However, the definite effect of eliminating SENV-H with IFN plus ribavirin needs to be further proved.

In conclusion, we found that nearly one-fifth Taiwanese patients with chronic hepatitis C are coinfected with SENV-H and coexistent SENV-H infection reveals apparent association with HCV genotype 1b but does not influence other clinico-pathological characteristics of HCV infection. After combination therapy with high dose IFN and ribavirin, two-thirds of patients achieve sustained HCV response and predictive factors for HCV SVR include HCV genotype non-1b, lower pretreatment HCV RNA levels and younger age. SENV-H is highly susceptible and does not affect the HCV response to combination therapy.

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References

Alter, M.J., Margolis, H.S., Krawczynski, K., Judson, F.N., Mares, A., Alexander, W.J., Hu, P.Y., Miller, J.K., Gerber, M.A., Sampliner, R.E., 1992. The natural history of community-acquired hepatitis C in the United States. N. Engl. J. Med. 327, 1899–1905.

Alter, M.J., Gallagher, M., Morris, T.T., Moyer, L.A., Meeks, E.L., Krawczynski, K., Kim, J.P., Margolis, H.S., 1997. Acute non-A–E hepatitis in the United States and the role of hepatitis G virus infection. N. Engl. J. Med. 336, 741–746.

Bowden, S., 2001. New hepatitis viruses: contenders and pretenders. J. Gastroenterol. Hepatol. 16, 124–131.

Chen, D.S., Wang, J.T., Chen, P.J., Wan, T.H., Sung, J.L., 1991. Hepatitis C virus infection in Taiwan. Gastroenterol. Jpn. 26 (Suppl. 3), 164–166.

Chuang, W.L., Chang, W.Y., Lu, S.N., Lin, Z.Y., Chen, S.C., Hsieh, M.Y., Wang, L.Y., You, S.L., Chen, C.J., 1993. The role of hepatitis C virus in chronic hepatitis B virus infection. Gastroenterol. Jpn. 28 (Suppl. 5), 23–27.

Dai, C.Y., Yu, M.L., Chuang, W.L., Lin, Z.Y., Chen, S.C., Hsieh, M.Y., Wang, L.Y., Tsai, J.F., Chang, W.Y., 2002a. Clinical characteristics and distribution of genotypes of TT virus infection in an HCV-hyperendemic township of an HBV-endemic country (Taiwan). J. Gastroenterol. Hepatol. 17, 1192–1197.

Dai, C.Y., Yu, M.L., Chuang, W.L., Sung, M.H., Lin, Z.Y., Chen, S.C., Hsieh, M.Y., Wang, L.Y., Tsai, J.F., Chang, W.Y., 2002b. Epidemiology and clinical significance of chronic hepatitis-related viruses infection in hemodialysis patients from Taiwan. Nephron 90, 148–153.

Dai, C.Y., Yu, M.L., Chen, S.C., Lin, Z.U., Hsieh, M.Y., Lee, L.P., Hou, N.J., Hsieh, M.Y., Wang, L.Y., Tsai, J.F., Chuang, W.L., Chang, W.Y., 2003. Clinical significance of TT Virus (TTV) infection in chronic hepatitis C patients with high dose interferon-alpha therapy in Taiwan: re-evaluated by using new set of TTV primers. Hepatol. Res. 27, 95–100.

Desmet, V.J., Gerber, M., Hoofnagle, J.H., Manns, M., Scheuer, P.J., 1994. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 19, 1513–1520.

Kao, J.H., Chen, W., Chen, P.J., Lai, M.Y., Chen, D.S., 2000. TT virus infection in patients with chronic hepatitis B or C: influence on clinical, histological and virological features. J. Med. Virol. 60, 387–392.

Kao, J.H., Chen, W., Chen, P.J., Lai, M.Y., Chen, D.S., 2002. Prevalence and implication of a newly identified infectious agent (SEN virus) in Taiwan. J. Infect. Dis. 185, 389–392.

Kao, J.H., Chen, W., Chen, P.J., Lai, M.Y., Chen, D.S., 2003. SEN virus infection in patients with chronic hepatitis C: preferential coinfection with hepatitis C genotype 2a and no effect on response to therapy with interferon plus ribavirin. J. Infect. Dis. 187, 307–310.

Knodell, R.G., Ishak, K.G., Black, W.C., Chen, T.S., Craig, R., Kaplowitz, N., Kiernan, T.W., Wollman, J., 1981. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1, 431–435.

Kobayashi, N., Tanaka, E., Umemura, T., Matsumoto, A., Iijima, T., Higuchi, M., Hora, K., Kiyosawa, K., 2003. Clinical significance of SEN virus infection in patients on maintenance haemodialysis. Nephrol. Dial. Transplant 18, 348–352.

Lai, M.Y., Kao, J.H., Yang, P.M., Wang, J.T., Chen, P.J., Chan, K.W., Chu, J.S., Chen, D.S., 1996. Long-term efficacy of ribavirin plus interferon alfa in the treatment of chronic hepatitis C. Gastroenterology 111, 1307–1312.

Lauer, G.M., Walker, B.D., 2001. Hepatitis C virus infection. N. Engl. J. Med. 345, 41–52.

McHutchison, J.G., Gordon, S.C., Schiff, E.R., Shiffman, M.L., Lee, W.M., Rustgi, V.K., Goodman, Z.D., Ling, M.H., Cort, S., Albrecht, J.K., 1998. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. N. Engl. J. Med. 339, 1485–1492.

Mushahwar, I.K., 2000. Recently discovered blood-borne viruses: are they hepatitis viruses or merely endosymbionts? J. Med. Virol. 62, 399–404.

Nishizawa, T., Okamoto, H., Konishi, K., Yoshizawa, H., Miyakawa, Y., Mayumi, M., 1997. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem. Biophys. Res. Commun. 241, 92–97.

Okamoto, H., Tokita, H., Sakamoto, M., Horikita, M., Kojima, M., Iizuka, H., Mishiro, S., 1993. Characterization of the genomic sequence of type V (or 3a) hepatitis C virus isolates and PCR primers for specific detection. J. Gen. Virol. 74, 2385–2390.

Poynard, T., Marcellin, P., Lee, S.S., Niederau, C., Minuk, G.S., Ideo, G., Bain, V., Heathcote, J., Zeuzem, S., Trepo, C., Albrecht, J., 1998. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 352, 1426–1432.

Rigas, B., Hasan, I., Rehman, R., Donahue, P., Wittkowski, K.M., Lebovics, E., 2001. Effect on treatment outcome of coinfection with SEN viruses in patients with hepatitis C. Lancet 358, 1961–1962.

Scheuer, P.J., 1991. Classification of chronic viral hepatitis: a need for reassessment. J. Hepatol. 13, 372–374.

Schröter, M., Laufs, R., Zöllner, B., Knödler, B., Schäfer, P., Sterneck, M., Fischer, L., Feucht, H.H., 2002. Prevalence of SENV-H viraemia among healthy subjects and individuals at risk for parenterally transmitted diseases in Germany. J. Viral. Hep. 9, 455–459.

Shibata, M., Wang, R.Y., Yoshiba, M., Shih, J.W., Alter, H.J., Mitamura, K., 2001. The presence of a newly identified infectious agent (SEN virus) in patients with liver diseases and in blood donors in Japan. J. Infect. Dis. 184, 400–404.

Simons, J.N., Leary, T.P., Dawson, G.J., Pilot-Matias, T.J., Muerhoff, A.S., Schlauder, G.G., Desai, S.M., Mushahwar, I.K., 1995. Isolation of novel virus-like sequences associated with human hepatitis. Nat. Med. 1, 564–569.

Tanaka, Y., Primi, D., Wang, R.Y., Umemura, T., Yeo, A.E., Mizokami, M., Alter, H.J., Shih, J.W., 2001. Genomic and molecular evolutionary analysis of a newly identified infectious agent (SEN virus) and its relationship to the TT virus family. J. Infect. Dis. 183, 359–367.

Umemura, T., Yeo, A.E., Sottini, A., Moratto, D., Tanaka, Y., Wang, R.Y., Shih, J.W., Donahue, P., Primi, D., Alter, H.J., 2001a. SEN virus infection and its relationship to transfusion-associated hepatitis. Hepatology 33, 1303–1311.

Umemura, T., Alter, H.J., Tanaka, E., Yeo, A.E., Shih, J.W., Orii, K., Matsumoto, A., Yoshizawa, K., Kiyosawa, K., 2001b. Association between SEN virus infection and hepatitis C in Japan. J. Infect. Dis. 145, 1246–1251.

Umemura, T., Alter, H.J., Tanaka, E., Orii, K., Yeo, A.E., Shih, J.W., Matsumoto, A., Yoshizawa, K., Kiyosawa, K., 2002. SEN virus: response to interferon alfa and influence on the severity and treatment response of coexistent hepatitis C. Hepatology 35, 953–959.

Wang, J.H., Lu, S.N., Wu, J.C., Huang, J.F., Yu, M.L., Chen, S.C., Chuang, W.L., 1999. A hyperendemic community of hepatitis B virus and hepatitis C virus infection in Taiwan. Trans. R. Soc. Trop. Med. Hyg. 93, 1–2.

Yu, M.L., Chuang, W.L., Dai, C.Y., Lu, S.N., Wang, J.H., Huang, J.F., Chen, S.C., Lin, Z.Y., Hsieh, M.Y., Tsai, J.F., Wang, L.Y., Chang, W.Y., 2001. The serological and molecular epidemiology of GB virus C/hepatitis G virus infection in a hepatitis C and B endemic area. J. Infect. 42, 61–66.