COINFECTIONS WITH HEPATITIS G AND/OR C VIRUS IN HEPATITIS B-RELATED CHRONIC LIVER DISEASE

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Abstract. Concurrent infections with HGV and/or HCV (HGV/HCV) were investigated in 196 patients with HBV-related chronic liver disease (115 chronic hepatitis, 31 liver cirrhosis, 50 hepatocellular carcinoma), and in 100 HBsAg carriers. Coinfections were detected in 18 (9.2%) patients with HGV (10) or HCV (5) or both agents (3), but in none of the HBsAg carriers. Patients with coinfection were more frequently exposed to blood transfusions (55.6% *vs* 5.6%) and also were more commonly anti-HBe positive. Serum levels of HBV-DNA were lower in patients with HCV coinfection than in those coinfected with HGV. Interferon was administered to 39 patients with chronic active hepatitis including 7 patients with HGV/HCV coinfection. Sustained clearance of HBV-DNA was observed in 10 (25.6%) patients who were solely infected with HBV. These patients were significantly younger and had much lower histological scores than non-responders. Patients with HCV coinfection had significantly higher pre-treatment histological scores than those without HCV. After interferon treatment, a significant reduction in histological scores was observed in all patients except those coinfected with HGV/HCV. None of the 7 patients with coinfection had sustained clearance of HBV-DNA or HCV-RNA, and only one had cleared HGV-RNA. These results suggest that parenteral exposure is a risk factor for HGV/HCV coinfection in chronic HBV infection. HGV infection shows no significant impact on chronic HBV infection. HCV coinfection appears to inhibit HBV replication, but causes more severe chronic hepatitis and increases resistance to interferon therapy.

INTRODUCTION

Viral hepatitis constitutes a major cause of chronic liver disease, such as cirrhosis and hepatocellular carcinoma (HCC). Especially in areas hyperendemic for hepatitis B virus (HBV), namely, Southeast Asia, China and sub-Saharan Africa (Lee, 1997), infection with this agent accounts for most cases of chronic liver disease. The risk of a chronic HBV carrier to develop HCC is approximately 100 times greater than that of an uninfected individual (Beasley, 1988). The probability to become a chronic carrier of HBV is highest among those either born to a carrier mother or infected early in life and hence, they are far more likely to proceed towards chronicity than those infected during adulthood (Popper *et al*, 1987).

In countries with low endemicity of HBV, as for example southern Europe and Japan, hepatitis C virus infection is most frequently found to proceed towards chronic liver disease (Colombo *et al*, 1989; Saito *et al*, 1990). HCV infections mainly occur among adults due to either blood transfusion or intravenous drug use applying non-sterile equipment. In more than 85% of the cases with acute infection, a tendency towards chronicity can be observed. Usually, chronic hepatitis C develops into HCC, in most cases preceded as well as accompanied by cirrhosis, within two to three decades (Tong *et al*, 1995).

Hepatitis G virus (HGV, GBV-C) has been discovered in 1995 almost simultaneously by investigators from Abbott Laboratories and Genelabs Technologies, Inc, who have identified this agent as belonging to the flaviviridae (Simons *et al*, 1995; Linnen *et al*, 1996). According to present data, HGV infection may persist in an asymptomatic state and only seldom cause acute hepatitis, which in the majority of patients does not develop into chronic liver disease (Alter *et al*, 1997; Pramoolsinsap, 1998).

All of the three viruses employing a parenteral route of transmission, double or triple coinfections can be detected with HBV, HCV and HGV. In patients with HCV-HGV coinfection, which appears to be rather common, the course of chronic hepatitis is closely related to HCV infection (Wang *et al*, 1996), whereas data on HGV coinfection in patients with HBV-related chronic liver disease are more limited and rather inconclusive.

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The purpose of the present study was to determine the significance of HCV and/or HGV coinfection in patients diagnosed with HBV-related chronic liver disease. Furthermore, these double or triple infections were evaluated as to clinical, biochemical, virological and histological response to interferon treatment.

MATERIALS AND METHODS

Population study

One hundred ninety-six patients with a mean age of 50.8 ± 13.8 years and a male: female sex ratio of 3.4: 1, diagnosed with chronic liver disease related to HBV infection were included in the study performed at Ramathibodi Hospital, Mahidol University, and at Phaya Thai Hospital, Bangkok, Thailand. At the onset, informed consent was obtained from each subject and the study was approved by the Research Committee of Mahidol University, Bangkok, Thailand.

Viral serology

Sera were obtained from all patients and kept at -70°C until further use. The sera of 100 HBsAg-positive but otherwise asymptomatic individuals who did not display any elevation of the liver enzymes served as controls. Moreover, the sera obtained from 200 healthy blood donors have been subjected to the same respective tests, the results of which have been published previously (Pramoolsinsap *et al*, 1998).

HBsAg, HBeAg and anti-HBe were determined by ELISA, Auszyme II (Abbott Laboratories, North Chicago, IL). Anti-HCV was detected by third generation ELISA (Abbott Laboratories, North Chicago, IL).

Blood biochemistry

Complete blood chemistry and liver function tests were performed using an automated chemical analyzer (Beckman Synchron CX5, BREA, CA) at the Central Laboratory, Ramathibodi Hospital.

Determination of HBV-DNA, HCV-RNA and HGV-RNA

Those samples positive for either HBsAg or anti-HCV were subjected to quantitative measurement of HBV-DNA, using the branched DNA signal amplification assay (bDNA Quantiplex™ HBV-DNA, Chiron Diagnostics, Emeryville, CA), and of HCV-RNA, using the second generation branched DNA signal amplification assay (Quantiplex™ HCV-RNA 2.0, Chiron Diagnostics, Emeryville, CA), respectively, according to the manufacturer's specifications. Serum HGV-RNA was determined by reverse transcription polymerase chain reaction (RT-PCR) as described previously (Pramoolsinsap *et al*, 1998).

HCV genotyping

HCV genotyping was performed according to Okamoto *et al* (1992).

Anti-viral therapy

Alpha interferon was administered to initially 42 patients, all of who suffered from chronic active hepatitis documented by biopsy and had proven positive for HBsAg, HBeAg, and HBV-DNA. Due to side effects of variable severity, only 39 patients remained for the entire duration of the study. Of those, all continuously displayed higher than two-fold ALT elevations for over six months. Recombinant interferon alpha-2b (Intron-A, Schering-Plough), 5 million units, was injected subcutaneously three times per week for the duration of 24 weeks. After initiation of the therapy, the patients were clinically assessed in the second and fourth week, thereupon every four weeks during treatment, and eventually at 2- to 3-months intervals for one additional year. Hematological and biochemical parameters, including serum ALT levels, were determined on a monthly basis in the course of the treatment, and upon its conclusion at 2- to 3-months intervals. The serology of HBV infection was reassessed after completion of the therapy and twice more after 6 and 12 months, respectively. Furthermore, those patients positive for double and/or triple infections, namely, HBV-HCV, HBV-HGV, or HBV-HCV-HGV, were also examined for residual HCV-RNA and/or HGV-RNA along with HBV-DNA after IFN-alpha treatment.

Therapeutic response to interferon was assessed in relation to the rates of seroconversion to anti-HBe, declining serum ALT levels and clearance of HBV-DNA. Patients who showed normalization of ALT levels, seroconversion of HBeAg to anti-HBe, clearance of HBV-DNA and/or histological improvement of chronic hepatitis for at least 12 months after completion of the therapy were considered sustained responders. Patients undergoing relapse had abnormal ALT levels, as well as reappearing HBeAg and/or HBV-DNA after an initial temporary response. Patients with persistently elevated ALT levels, detectable HBeAg and/or HBV-DNA during and after completion of interferon treatment were considered non-responders.

Histopathology

All patients selected for interferon treatment were subjected to liver biopsy before initiation of therapy and subsequently at 6-12 months after completion of the therapy. The biopsy was performed using a disposable Menghini needle (Hepafix). Liver biopsy specimens were fixed in 10% formalin. Hepatic histopathological findings were interpreted by a pathologist in-

dependent of clinical and biochemical data, according to the criteria described by Knodell *et al* (1981).

Statistical analysis

The clinical and biochemical data are presented as mean ± SD. Comparisons between groups of liver disease or between patients with and without HGV/HCV coinfection were made by Student's *t*-test, chi-square test, or Fisher's exact method for parametric data and by the Mann-Whitney U test for nonparametric data. A p-value of 0.05 or below indicated statistical significance.

RESULTS

Demographic data

The study comprised 196 patients with HBV-related chronic liver disease whose further details have been depicted in Table 1. It should be noted that the group of patients with CH was significantly younger than the cirrhosis and HCC group, respectively ($p < 0.001$). The mean age of 100 asymptomatic HBsAg carriers serving as controls was 40.1 ± 12.6 years and their sex ratio was 1:1.

The majority of the patients proved positive for either HBeAg or anti-HBe. Between the 3 groups, the prevalence of HBeAg was highest in patients with CH ($p < 0.001$), whereas the majority of patients in the other two groups were positive for anti-HBe (Table 1). Previous parenteral exposure was confirmed in 20 patients and there were no significant differences as to exposure rate among the different types of liver disease (Table 1). Patients with HCC clearly had the lowest HBV-DNA levels ($p \leq 0.015$) and the highest alpha-fetoprotein levels ($p < 0.001$). The other laboratory findings including ALT levels were not significantly different within the 3 patient groups (Table 2).

Prevalence of HGV/HCV coinfections

Coinfection with HGV/HCV was detected in 18 patients (9.2%) but not in asymptomatic HBsAg carriers. These patients were coinfected with either HGV (5.1%), HCV (2.6%) or both (1.5%) (Table 1,3,4). The overall mean age of patients with coinfection as well as their sex ratio were similar as those of patients solely infected with HBV. Double and triple infections were found in all types of liver disease and there were no significant differences between the 3 groups regarding HGV/HCV coinfection rates. Of the 8 patients with HCV coinfection, 5 were genotype II and 3 genotype III.

Significance of HGV/HCV coinfection

Patients with HCV/HGV coinfection were more

Table 1
Characteristic data of 196 patients with HBV-related chronic liver disease.

Characteristic data	Groups of patients			
	Chronic hepatitis	Cirrhosis	HCC	Total
No. of patients (%)	115 (58.7%)	31 (15.8%)	50 (25.5%)	196 (100%)
Age (mean±SD, yr)	40.2 ± 12.2*	54.0 ± 9.8	53.8 ± 11.0	50.8 ± 13.8
Male, n (%)	89 (77.4%)	21 (67.7%)	42 (84.0%)	152 (77.6%)
HBeAg positive, n (%)	62 (53.9%)	6 (19.4%)	7 (14.0%)	75 (38.3%)
Male/Female ratio	4.6 / 1	1 / 1	2.5 / 1	3.7 / 1
Anti-HBe positive, n (%)	45 (39.1%)	25 (80.6%)	43 (86.0%)	113 (57.7%)
Male/Female ratio	2.8 / 1	2.6 / 1	6.2 / 1	3.5 / 1
HGV-RNA positive, n (%)	7 (6.1%)	4 (12.9%)	2 (4.0%)	13 (6.6%)
Male/Female ratio	6 / 1	3 / 1	1 / 1	3.3 / 1
Anti-HCV positive, n (%)	6 (5.2%)	0	2 (4.0%)	8 (4.1%)
Male/Female ratio	4 / 2		1 / 1	5 / 3
HBV-DNA; MEq/ml	167.8	36.1	0.7*	41.7
(median; ranges)	0.7– >5,000	8.6 – 676.1	0.7–8.64	0.7– >5,000
Parenteral exposure; n				
Post-transfusion	5	2	1	8
IV drug users	1	0	0	1
Tattoos/Acupuncture	2	7	2	11

* = statistically difference from the other groups; $p < 0.05$

Table 2
Baseline biochemical results (mean±SD) of 196 patients with HBV-related chronic liver diseases.

Parameters (normal values)	Groups of patients		
	Chronic hepatitis	Cirrhosis	HCC
Hemoglobin (g%)	14.6 ± 1.6	13.2 ± 1.8	12.7 ± 2.2
Platelet count	207.8 ± 62.1	133.2 ± 47.4	159.8 ± 58.4
ALT (6-36 U/l)	198.1 ± 82.2	72.3 ± 36.2	62.4 ± 28.1
AST (14-33 U/l)	142.8 ± 73.8	78.0 ± 31.0	108.2 ± 85.1
GGT (5-38 U/l)	77.9 ± 64.7	99.5 ± 63.2	154.9 ± 104.1
AP (20-90 U/l)	80.9 ± 33.4	115.5 ± 91.7	189.7 ± 144.6
Albumin (32-55 g/l)	44.4 ± 5.6	38.5 ± 8.9	36.5 ± 11.1
γ-globulin (28 g/l)	31.2 ± 10.2	33.9 ± 10.1	34.2 ± 7.6
Bilirubin (3.4-17.1 μmol/l)	21.0 ± 9.8	59.9 ± 41.9	61.6 ± 47.9
Prothrombin time (second)	15.2 ± 2.7	16.4 ± 4.0	16.4 ± 3.8
Alpha-fetoprotein (median, ranges; ng/ml)	5.0 1.5 – 320	4.7 1.5 – 11.8	276* 75 – 108,870

ALT = Alanine aminotransferase, AST = Aspartate aminotransferase GGT = γ-glutamyl transpeptidase , AP = Alkaline phosphatase; * = statistically difference from the other groups, $p < 0.05$

Table 3
Baseline data in patients with HBV-related chronic liver disease with and without HGV co-infection.

Baseline data	HGV-RNA status		
	Positive	Negative	Total
Number of patients (%)	13 (6.6%)	183 (93.4%)	196 (100%)
Number of male; n (%)	10 (76.9%)	143 (78.1%)	153 (78.1%)
Age (mean± SD, years)	50.3 ± 11.0	44.3 ± 13.3	44.7 ± 13.2
ALT (mean±SD, U/l)	133.4 ± 67.3	159.5 ± 95.2	158.0 ± 103.2
AST (mean±SD, U/l)	156.0 ± 102.7	125.7 ± 69.4	127.5 ± 98.0
GGT (mean±SD, U/l)	112.8 ± 83.8	96.1 ± 61.0	96.8 ± 50.7
AP (mean±SD, U/l)	151.1 ± 124.9	102.1 ± 78.9	104.9 ± 84.1
HBeAg positive; n (%)	2 (15.4%)	73 (39.9%)	75 (38.3%)
Anti-HBe positive; n (%)	9 (69.2%)	104 (56.8%)	113 (57.7%)
Anti-HCV positive; n (%)	3 (23.1%)	5 (2.7%)	8 (4.1%)
HBV-DNA (median, ranges; MEq/ml)	219.5 52.3 – 386.7	40.9 0.7 – >5,000	41.7 0.7 – >5,000
Parenteral exposure; n			
- Post-transfusion	4	4	8
- IV drug users	0	1	1
- Tattoos	2	9	11

ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, GGT = γ-glutamyl transpeptidase, AP = Alkaline phosphatase

common (23%) than those exhibiting only HCV coinfection (2.7%; $p < 0.001$). Between patients coinfected with HGV or HCV, there were no significant differences regarding age, sex ratio, prevalence of HBeAg or anti-HBe, duration of blood transfusion or biochemical data (Table 3, 4). The majority of patients with HGV/HCV coinfection were anti-HBe positive (72.2%) and similarly, all but one of the HCV coinfected patients were anti-HBe positive. The ratio of patients positive for anti-HBe/HBeAg was higher among patients with HGV/HCV coinfection (4.3 : 1) than in those solely infected with HBV (1.4 : 1 ; $p = 0.06$). Previous parenteral exposure including blood transfusion, intravenous drug abuse, or tattoos were sig-

Table 4
Characteristics baseline data of patients with double or triple viral hepatitis infections.

Baseline data	Groups of patients		
	HBV + HCV	HBV + HGV	HBV + HCV + HGV
Number of patients	5	10	3
Age (mean ± SD, yr)	52.2 ± 16.5	49.8 ± 11.8	49.3 ± 8.1
No. of male/ female	2 / 3	7 / 3	3 / 0
Liver disease, n	4 CH, 1 HCC	4 CH, 4 CIR, 2 HCC	2 CH, 1 HCC
HBeAg positive, n	1	2	0
Anti-HBeAg positive, n	4	6	3
Transfusion, n	4	2	2
Duration of Tx (mean±SD, yr)	10.0 ± 2.7	8.0 ±1.0	13.0 ± 1.4
ALT (mean±SD, U/l)	119.2 ± 63.4	125.3 ± 53.0	101.3 ± 16.2
AST (mean±SD, U/l)	116.8 ± 50.5	135.9 ± 60.2	84.7 ±14.1
GGT (mean±SD, U/l)	74.0 ± 55.4	41.0 ± 20.2	74.0 ± 45.3
HBV-DNA (median, ranges, MEq/ml)	0.5 (0.5-52.3)	15.2(0.7-4,400)	5.2 (3.3-6.5)

CH = Chronic hepatitis; CIR = Cirrhosis, HCC = Hepatocellular carcinoma; Tx = transfusion

nificantly more common among patients coinfected with HGV (46.2%) or HGV/HCV (55.6%) than among those only infected with HBV (5.6%; P< 0.001). There were no significant differences between patients with and without HGV or HGV/HCV coinfections as to HBV-DNA levels or other laboratory data (Table 3).

HBV-DNA and HCV-RNA in patients with coinfection

Serum levels of HBV-DNA in the 8 patients with HCV coinfection (0.5-52.3 MEq/ml) were lower than those of their HCV-RNA (4.2-76.7 MEq/ml), although these differences were not statistically significant (p = 0.07). Also, the levels of HBV-DNA were lower in patients with HCV than in those with only HGV coinfection (0.7-4,400 MEq/ml). Yet, these differences were not significant (p = 0.21).

Response to interferon therapy

Interferon was administered to 39 patients with chronic active hepatitis (Table 5) including 7 patients with HGV/HCV coinfection. All patients completed 24 weeks of therapy and were subsequently followed for at least 12 months. The majority of the patients (56.4%) responded to the treatment, but only 10 (25.6%) were sustained responders. The mean age of the sustained responders was similar to that of patients with relapse, but significantly below that of the non-responder group (p = 0.012). Among the 3 groups of therapy responders, there was no significant difference as to the prevalence of viral serology (HBeAg, anti-HBe), HGV-RNA or HCV-RNA, biochemical data, or baseline HBV-DNA levels (Table 5).

The 7 patients with coinfections undergoing therapy were positive for either HGV-RNA (n = 2), HCV-RNA (n = 3), or both (n = 2). Upon completion of the treatment, none of these patients showed sustained clearance of HBV-DNA or HCV-RNA and only one patient had cleared HGV-RNA. Neither of the 2 patients with triple infections did respond to the therapy, but remained positive for HBV-DNA, HCV-RNA and HGV-RNA.

Histological changes in response to interferon treatment

According to the Knodell score, all baseline histological parameters, except fibrosis, underwent a significant decrease in the sustained responders compared to the remaining patients (p ≤ 0.042; Table 6). Total histological scores above 10 were found in the majority of non-responders or relapse cases, but in none of the sustained responders (p = 0.005). Upon conclusion of therapy, the total histological scores of all treated patients had decreased (p < 0.001; Table 6). This significant decline in histological parameter scores could be observed in all 3 responder groups, with the exception of fibrosis, the decrease of which was significant only among the sustained responders (p = 0.018).

On comparison of patients with and without HGV/HCV coinfection, the baseline histological scores of focal necrosis (3.3 ± 0.5 *vs* 1.4 ± 0.9) and periportal bridging necrosis (3.6 ± 0.8 *vs* 2.3 ± 1.2), respectively, were significantly higher among the former (p ≤ 0.008)

Table 5
Baseline data according to types of therapeutic responses in 39 interferon treated patients.

Parameters	Type of therapeutic responses		
	Sustained responders	Relapse	Non-responders
No. of patients (%)	10 (25.6%)	11 (28.2%)	18 (46.2%)
Age (mean± SD, yr)	32.3 ± 11.6*	37.4 ± 6.7	44.4 ± 11.8
No. of male/ female	6 / 4	10 / 1	16 / 2
HBeAg positive, n	10	9	15
Anti-HBe positive, n	0	2	1
HGV-RNA positive, n	0	1	2
Anti-HCV positive, n	1	0	3
ALT (mean±SD, U/l)	231.9 ± 106.1	252.8 ± 136.7	214.7 ± 92.8
AST (mean±SD, U/l)	141.1 ± 44.8	184.5 ± 110.2	183.9 ± 106.3
GGT (mean±SD, U/l)	47.3 ± 29.0	79.1 ± 57.3	137.2 ± 88.8
HBV-DNA (median,	430.25	366.70	73.45
ranges, MEq/ml)	0.70 – 3,351.0	0.70 – >5,000	0.70 - 2,610

* = statistically difference from nonresponder group, p = 0.012.

Table 6
Histological scores before and after treatments in 39 patients.

Parameters of histological scores	Type of therapeutic responses		
	Sustained responders	Relapse	Non-responders
Before treatment			
Portal inflammation	1.8 ± 0.79	2.45 ± 0.82	3.11 ± 0.83
Focal necrosis	1.2 ± 0.63	1.91 ± 0.83	2.06 ± 1.30
Periportal bridging necrosis	1.5 ± 0.71	2.18 ± 1.08	3.22 ± 1.11
Fibrosis	2.3 ± 1.57	3.18 ± 1.17	3.00 ± 1.24
Total	6.8 ± 1.75	9.54 ± 2.34	11.72 ± 2.67
After treatment			
Portal inflammation	1.0 ± 0.82	1.54 ± 0.93	1.61 ± 1.46
Focal necrosis	0.5 ± 0.53	0.82 ± 0.60	2.72 ± 1.53
Periportal bridging necrosis	0.9 ± 0.32	1.00 ± 1.09	2.89 ± 1.23
Fibrosis	1.1 ± 0.74	2.54 ± 1.21	2.72 ± 1.53
Total	3.5 ± 0.53	5.91 ± 1.37	10.0 ± 0.73

but the scores for portal inflammation and fibrosis were similar ($p \geq 0.69$). When comparing patients with and without HCV coinfection, the former showed significantly higher baseline scores for all histological parameters ($p \leq 0.010$), except for fibrosis ($p = 0.18$). Upon conclusion of the therapy, no significant decline in the histological scores became apparent in patients with HCV or HCV/HGV coinfections (13.3 ± 2.6 *vs* 13.0 ± 3.1; $p = 0.17$).

DISCUSSION

Simultaneous infections with HBV along with HGV and/or HCV (HCV/HGV) have been reported on a worldwide scale, particularly in areas hyperendemic for HBV. Anti-HCV has been detected in between 10 and 15% of HBsAg positive patients with chronic liver disease (Sato *et al*, 1994; Ohkawa *et al*, 1994; Crespo *et al*, 1994; Fattovitch *et al*, 1991; Chen *et al*, 1990), including hepatocellular carcinoma. (Benvegnu *et al*, 1994; Fong *et al*, 1991). Whereas coinfection with HGV has been reported to occur ubiquitously in 3 to 11% of patients chronically infected with HCV, the HGV coinfection rate among patients with chronic HBV infection remains to be elucidated.

In the present study, 9.2% of the patients diag-

nosed with HBV-related chronic liver disease were found coinfected with either HGV or HCV. Yet, none of the asymptomatic HBsAg carriers showed coinfection with either virus. These findings correspond to a report from Hong Kong where HCV/HBV coinfection is common among patients with chronic liver disease, yet anti-HCV could be detected in a mere 0.7% of HBsAg carriers (Lau *et al*, 1998). Contrasting patients only infected with HBV, our patients with HCV/HGV coinfection were much more frequently exposed to parenteral transfusion. Although all blood banks in Thailand are routinely screened for both HBsAg and anti-HCV, we found 6 of 8 patients with HCV coinfection had received emergency blood transfusion (18-21 units) in rural hospitals following near-fatal accidents. Furthermore, anti-HCV was more prevalent among patients positive for HGV-RNA compared to those solely infected with HBV. These data suggest parenteral exposure to constitute a crucial risk factor with respect to coinfection with these hepatitis viruses.

The infection rate determined for HGV in the present study was slightly above that found in a previous study among HCV-related chronic liver disease patients (Pramoolsinsap *et al*, 1998) in that now, HGV coinfection was found among all age groups, as well as all types of chronic liver disease, including hepatocellular carcinoma. These results are contrary to our previous findings in HCV-related chronic liver disease where HGV coinfection was exclusively confounded to chronic hepatitis patients between 30 and 40 years of age (Pramoolsinsap *et al*, 1998). Unlike HCV, HBV might inhibit spontaneous clearance of HGV, which seems to depend on the patient's age and on the duration of infection. (Francesconi *et al*, 1997; Diamentis *et al*, 1997) Hence, particularly as these findings may be incidental further studies are required in order to elucidate the interaction of HGV with HBV and HCV. In the present study, as was reported for HCV-related chronic liver disease (Pramoolsinsap *et al*, 1998; Kao *et al*, 1998), HGV likewise had no significant impact on the course of HBV-related chronic liver disease.

The potential impact of coinfection with hepatitis viruses is of major concern, especially HBV and HCV infections which both can progress towards hepatocellular carcinoma (Hino and Kajino, 1994). Also, HBV/HCV coinfection can increase the risk of fulminant hepatitis (Chu *et al*, 1994) and therefore tends to induce a more severe and progressive course of disease (Chu *et al*, 1994; Wu *et al*, 1994). However, the interaction between both viruses in coinfection has remained controversial. Various studies have indicated HCV to inhibit or interfere with HBV replication, whereas other studies have shown a reciprocal suppression of HCV replication by HBV (Sato *et al*, 1994; Ohkawa *et al*, 1994; Colombari *et al*, 1993). It has been suggested that the virus having infected more recently tends to suppress the preexisting virus (Wu *et al*, 1994). Direct inhibition of HBV replication by HCV has been shown in animal models and cell culture systems (Sato *et al*, 1994; Shih *et al*, 1993). Indirect evidence of the inhibitory effect of HCV has been provided by several studies where patients with HBV/HCV coinfection were found to display either weaker HBV-DNA polymerase activity (Sato *et al*, 1994; Crespo *et al*, 1994; Fong *et al*, 1991) or lower titers of HBsAg and anti-HBc (Crespo *et al*, 1994) when compared with patients only infected with HBV.

In the present study, all but one patient coinfected with HCV were anti-HBe positive and the ratio of anti-HBe to HBeAg was three fold higher in patients with dual or triple infection than in those solely infected with HBV. The average HBV-DNA levels determined in the 8 patients coinfected with HCV were lower than the HCV-RNA levels and were also lower in comparison to the HBV-DNA levels detected in patients only coinfected with HGV. Although, due to the limited number of cases, these differences were not statistically significant, these findings correspond with the majority of studies suggesting HCV coinfection to interfere with HBV replication and hence to facilitate seroconversion from HBeAg to anti-HBe (Sato *et al*, 1994; Ohkawa *et al*, 1994; Crespo *et al*, 1994; Fattovitch *et al*, 1991; Colombari *et al*, 1993).

The prognosis of HBV/HCV dual infection is similarly controversial. Many studies have indicated that compared with single hepatitis virus infection, dual HBV/HCV infection causes more severe liver disease (Liaw *et al*, 1994, 1995; Villari *et al*, 1995; Chu *et al*, 1994; Crespo *et al*,1994; Ohkawa *et al*, 1994; Sato *et al*, 1994; Wu *et al*, 1994; Sheen *et al*, 1992; Fattovitch *et al*, 1991; Fong *et al*, 1991) with an inherent higher risk to proceed towards cirrhosis and hepatocellular carcinoma (Benvegnu *et al*, 1994; Crespo *et al*, 1994; Fong *et al*, 1991). Contrasting that, other studies have suggested HCV to suppress or even eliminate HBV, thereby resulting in histological and biochemical normalization (Fong *et al*, 1991; Sheen *et al*, 1992), or at least in neither exacerbation nor regression of histological alterations (Colombari *et al*, 1993).

In the present study, HCV coinfections were discovered in all liver disease groups including hepatocellular carcinoma. Upon comparison of patients with and without HGV/HCV coinfections, no significant differences could be discerned regarding serum levels

of ALT or HBV-DNA. However, the histological scores in patients subjected to interferon therapy were significantly higher in those with HCV or HGV/HCV coinfections than in those solely infected with HBV. These findings suggest HCV coinfection to cause more severe HBV-related hepatic cell damage, despite its inhibitory effect on HBV replication. It has been suggested that in HBV/HCV coinfections HCV is the more virulent agent (Tsai *et al*, 1995) which might usurp the role of HBV and act as the major cause of continuing hepatitis (Liaw *et al*, 1994).

The response to interferon therapy is variable in patients chronically infected with HBV. Hence, in the present study, despite a significant reduction in the overall histological scores, only 25% of the patients treated showed sustained clearance of HBV-DNA. Either those patients in the third decade of life or the ones with histological scores below 10 were found to react favorably to the therapy. The response of patients with coinfections was unsatisfactory as none of our 7 patients with HGV/HCV coinfection showed sustained clearance of either HBV-DNA or HCV-RNA and as HGV-RNA was cleared in merely one patient. Unlike patients solely infected with HBV, those with HCV or HGV/HCV coinfections did not exhibit any significant alterations in their histological scores upon completion of therapy. Corresponding with the results of Liaw, these findings suggest HCV coinfections to enhance resistance to interferon therapy (Liaw *et al*, 1995). Hence, a protracted course of interferon treatment ought to be considered in patients doubly infected with HBV and HCV.

The results of the present study suggest coinfection with HGV/HCV to be common among patients with HBV-related chronic liver disease, in particular those with previous parenteral exposure. HGV coinfection does not appear to interfere with either clinical severity or disease progression. HCV coinfection on the one hand seems to inhibit HBV replication, yet on the other to cause more severe chronic hepatitis and to increase resistance to interferon therapy. The applied 6-month course of interferon treatment has resulted in a sustained response among merely one fourth of the patients and the drug has been found more effective among either patients of a relatively young age group or those with low histological scores.

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