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World J Gastroenterol 2003;9(9):2012-2016
World Journal of Gastroenterology
 ISSN 1007-9327

• VIRAL HEPATITIS •

Spontaneous viral clearance after 6-21 years of hepatitis B and C viruses coinfection in high HBV endemic area

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Received: 2003-04-10 **Accepted:** 2003-05-19

Abstract

AIM: To investigate the clinical and virological course of coinfection by hepatitis B virus (HBV) and hepatitis C virus (HCV) in China.

METHODS: We enrolled 40 patients with chronic HBV and HCV coinfection (Group BC), 16 patients with chronic HBV infection (Group B) and 31 patients with chronic HCV infection (Group C). They infected HBV and/or HCV during 1982 to 1989. Sera of all the 87 patients were collected in 1994 and 2002 respectively. We detected biochemical and virologic markers and serum HBV DNA and HCV RNA levels of all the patients. B-type ultrasound detection was performed in some patients.

RESULTS: In Group BC, 67.5 % of the patients cleared HBsAg, and 92.5 % of the patients cleared HBeAg. The clearance rate of HBV DNA was 87.5 %. There was no significant difference of HBV clearance between Group BC and Group B. In Group BC, 85.7 % of males and 47.4 % of females cleared HBV, and males were easier to clear HBV (χ^2=6.686, P=0.010). Such a tendency was also found in Group B. The clearance rate of HCV RNA in Group BC was 87.5 %, significantly higher than that in Group C (χ^2=22.963, P<0.001). Less than 40 % of the patients in all groups had elevated liver enzyme values. The highest value of alanine aminotransferase (ALT) was 218 u/L (normal range for ALT is 0-40 u/L). In most patients the ultrasonogram presentations changed mildly.

CONCLUSION: The clinical manifestations of patients with HBV/HCV coinfection are mild and occult. High clearance rate of HBV and easy to clear HBV in male patients are the characteristics of HBV infection in adults in China. HBV can inhibit HCV replication, but no evidence has been found in our data that HCV suppresses HBV replication.

Fan CL, Wei L, Jiang D, Chen HS, Gao Y, Li RB, Wang Y. Spontaneous viral clearance after 6-21 years of hepatitis B and C viruses coinfection in high HBV endemic area. *World J Gastroenterol* 2003; 9(9): 2012-2016
http://www.wjgnet.com/1007-9327/9/2012.asp

INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most common causes of chronic liver diseases worldwide. Both viruses could induce chronic hepatitis, which may progress to cirrhosis and eventually to hepatocellular carcinoma (HCC). Because HBV and HCV share similar transmission routes, coinfection seems to be frequent. Seroprevalence studies have shown that coinfection of HCV is detected in around 10 % to 15 % of patients with chronic HBV infection, although the prevalence may vary from country to country[1-3]. Earlier studies in chimpanzees showed that replication of pre-existing HBV was inhibited by superinfection of NANB hepatitis virus (HCV)[4]. Some clinical observations indicate that HCV may inhibit HBV expression and even act as the major cause of chronic hepatitis[5-8]. And it was also reported that HBV might suppress replication of HCV[9-12]. The histopathological findings and clinical outcome in some of these cases were contradictory. Some researchers found that HBV and HCV coinfection could significantly increase the risk of development of fulminant hepatitis and also cirrhosis and HCC[2,13-16]. But others showed neither exacerbation nor diminution of histopathological changes in patients with HBV/HCV coinfection[17]. Coinfection does not play an important role in the development of HCC[18,19]. Furthermore, in patients with HBV and HCV coinfection after orthotopic liver transplantation, presence of HCV may improve their clinical outcome as compared with HBV infection alone[20].

Our previous study showed that the history of patients infected with HCV in China was different from that in Western countries[21]. At the same time, we enrolled a group of patients with chronic HBV and/or HCV infection during 1982 to 1988. Sera of all the 87 patients were collected in 1994 and 2002 respectively. We analyzed the biochemical and virologic markers and serum HBV DNA and HCV RNA levels in patients with chronic coinfection and compared the results with the patients with single HBV or HCV infection. The clinical and virological course of coinfection of HBV and HCV in China was investigated.

MATERIALS AND METHODS

Patients

Eighty seven patients with chronic HBV and/or HCV infection were native individual blood donors in Hebei Province of China, who had the history of drawing plasma from the blood and transfusion back of the blood cells during 1982-1989. The serum samples of these patients were collected in 1994 and 2002, and stored without thawing at -70 ℃. We detected the serological markers of HBV and HCV in all the patients in 1994 and 2002 respectively. All the patients were divided into 3 groups: Group BC (HBV and HCV coinfected group including 40 cases), Group B (single HBV infected group including 16 cases), and Group C (single HCV infected group including 31 cases). There was no significant difference in the number of patients, age and sex distribution, and duration of infection among the groups (Table 1). No patient had received treatment of immunosuppressors or antiviral agents such as interferon. And all the patients were negative for anti-HDV and anti-HIV. None of them had a history of auto-immune disease, and alcohol abuse.

Table 1 Clinical data for patients with HBV and/or HCV infection

	Group BC	Group B	Group C	Statistical value
Number of patients	40	16	31	
Sex (male/female)	21/19	6/10	15/16	χ^2=1.030, *P*>0.05
Age (y)[a]	39±5 (29-50)	40±6 (31-53)	39±3 (32-44)	F=0.48, *P*>0.05
Time of infection (y)[a]	16±5 (14-21)	16±5 (14-21)	17±4 (14-21)	F=2.27, *P*>0.05
Serological markers (1994)				
HBsAg +	34 (85.0 %)	16 (100 %)	0	
HBeAg +	11 (27.5 %)	7 (43.8 %)	0	
HBVDNA +	37 (92.5 %)	14 (87.5 %)	0	
HCVRNA +	11 (27.5 %)	0 (0 %)	19 (61.3 %)	
Anti-HCV	39 (97.5 %)	0 (0 %)	31 (100.0 %)	

[a]Data were expressed as mean ±SD (range).

Serological examination

Serum anti-HBs, anti-HBe, anti-HBc and HBeAg were detected by commercially available enzyme-linked immunosorbent assay (ELISA) kits (RADIM, Italia). HBsAg, anti-HIV and anti-HDV were tested by ELISA kits (Organon Teknika China Ltd). Testing of anti-HCV was performed by the third-generation of ELISA kits (Ortho Diagnostic Systems Inc. NJ). Liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) were determined using HITACHI 7170 biochemistry analyzer. Serum AFP was measured by radioimmunoassay. A value greater than 20 ng/ml was considered abnormal.

Quantitative detection of HBV DNA and HCV RNA

Serum HBV DNA and HCV RNA concentrations were determined by a programmable high-speed thermal cycler (Light Cycler II; Roche Diagnostics, Mannheim, Germany) for fluorescence quantitative polymerase chain reaction (FQ-PCR) using a commercially available kit (PG Biotech Co., Ltd., China). For HBV DNA FQ-PCR, the detection limit of the method was 500 genome copies/ml. For HCV RNA, the sensitivity limit of the method was about 1 000 genome copies/ml.

Statistical analysis

All the data were presented as the mean ±SD. χ^2 test or Fisher' s exact test was used for categorical variables. The statistical package for social sciences (SPSS, Chicago, IL), version 10.0, was used for statistical analyses. Significance was set at a *P* value of less than 0.05.

RESULTS

Spontaneous clearance of HBV (Table 2)

HCV did not influence the clearance of HBV in Group BC Persistent negative HBsAg and HBV DNA in serum and liver enzyme was considered as a criterion for HBV clearance[22, 23]. In Group BC, 27 of the 40 patients (67.5 %) cleared HBV, and in Group B, 7 of the 16 patients (43.8 %) cleared HBV. No significant difference was found between the two groups (χ^2=2.703, *P*=0.100) (Table 2). It was suggested that HCV had no effect on the elimination of HBV.

Spontaneous clearance of HBV in the course of chronic HBV infection Forty patients with coinfection of HBV and HCV had acquired the viruses for 6 to 13 years. Six of them (15 %) cleared HBsAg, 29 of them (72.5 %) cleared HBeAg, and 3 of them (7.5 %) cleared HBV DNA spontaneously in 1994 (Table 2). While in Group B, all the patients were positive for HBsAg and HBV DNA, and 10 of them (62.5 %) cleared HBeAg in 1994. In Group BC, 27 patients (67.5 %) cleared HBsAg, 37 patients (92.5 %) cleared HBeAg, and 35 patients (87.5 %) cleared HBV DNA in 2002. No significant difference was found between the two groups (Table 2). It was suggested that HBV could be cleared even after 6 years of infection in adults.

Influence of sex and serum virus load on the clearance of HBV In Group BC, 85.7 % (18/21) of the males cleared HBV, but only 47.4 % (9/19) of the malas cleared HBV. The HBV clearance rate of males was significantly higher than that of females (*P*<0.05). In Group B, the spontaneous HBV clearance rates were 66.7 % (4/6) and 30 % (3/10) in males and females respectively, but no statistical difference was found between them. It showed that males seemed easier to clear HBV (Table 2). According to the criteria of HBV clearance[22,23], 10 of the 16 patients (62.5 %) in Group BC with a high HBV DNA level had cleared HBV in 2002. Among the 24 patients who had low HBV DNA level, 17 patients (70.8 %) cleared HBV. There was no significant difference between the high and low HBV DNA level groups in Group BC (*P*>0.05). In Group B, 3 of the 9 patients (33.3 %) with a high HBV DNA level cleared HBV in 2002. While in 7 patients whose serum HBV level in 1994 was lower than 10^5 genomic copies/ml, 4 patients (57.1 %) cleared HBV (*P*>0.05) (Table 2). It was suggested that the level of virus load had no effect on the clearance of HBV infection.

Table 2 Clearance of serum HBV markers in Group BC and Group B

	Group BC (*n*=40, %)		Group B (*n*=16, %)	
HBsAg clearance				
Until 1994	6	(15%)	0	(0%)
Until 2002	27	(67.5%)	8	(50%)
HBeAg clearance/seroconversion				
HBeAg clearance				
Until 1994	29	(72.5%)	10	(62.5%)
Until 2002	37	(92.5%)	14	(87.5%)
HBeAg seroconversion	22	(55.5%)	12	(75%)
HBV DNA clearance				
Until 1994	3	(7.5%)	2	(12.5%)
Until 2002	35	(87.5%)	13	(81.2%)
HBV clearance	27	(67.5%)	7	(43.8%)
Male	18	(85.7%)[a]	4	(66.7%)
Female	9	(47.4%)[b]	3	(30%)
Low HBV DNA level group				
(<10^5 copies/ml)	17	(70.8%)	4	(57.1%)
High HBV DNA level group				
(≥10^5 copies/ml)	10	(62.5%)	3	(33.3%)

Note. [a] *vs.* [b]χ^2=6.686, *P*=0.010.

Spontaneous clearance of HCV RNA

In Group BC, 29 of the 40 patients cleared HCV RNA (72.5 %) in 1994. Thirty five patients (87.5 %) cleared HCV RNA in 2002. Two patients who were negative for HCV RNA in 1994 became positive in 2002. In Group C, 12 of the 31 patients cleared HCV RNA (38.7 %) in 1994, and 10 patients (32.3 %) cleared HCV RNA in 2002. Three patients who were formerly

negative for HCV RNA became positive in 2002. In 1994 and 2002, the clearance rate of HCV RNA in Group BC was higher than that in Group C (P<0.01) (Table 3).

Table 3 HCV RNA clearance rate in Group BC and Group C

	Group BC (n=40, %)	Group C (n=31, %)	Statistics value
HCV RNA clearance			
Until 1994	29 (72.5%)[a]	12 (38.7%)[b]	χ^2=8.173, (a *vs.* b) P<0.05
Until 2002	35 (87.5%)[c]	10 (32.3%)[d]	χ^2=22.963, (c *vs.* d) P<0.001
Male	17 (80.9%)	3 (20%)	
Female	18 (94.7%)	7 (43.8%)	

Relationship between HBV DNA and HCV RNA

Forty patients with HBV and HCV coinfection were dovided into 3 groups based on their serum HBV DNA levels in 1994. Within each of the groups, the patients were reclassified based on their serum HCV RNA viral load in 1994 (Table 4). Neither inverse nor positive correlation was found between HBV DNA and HCV RNA levels in the coinfected patients. However, detection of the sera of coinfected patients collected in 2002 showed that one who was positive for HBV DNA in 1994 was negative for HCV RNA. On the contrary, one who was positive for HCV RNA in 1994 was negative for HBV DNA. The fact that HBV DNA and HCV RNA could not be found in the same patient at the same time suggested that HBV and HCV were cleared one by one.

Table 4 Relationship of serum HBV and HCV load in 1994

HCV RNA (copies / ml)	HBV DNA(copies / ml)			Total amount
	$<10^3$	10^3-10^5	$\geqslant 10^5$	
$<10^3$	2	14	12	28
10^3-10^5	1	5	3	9
$\geqslant 10^5$	0	2	1	3
Total amount	3	21	16	40

P=0.918.

Table 5 Type B ultrasonic presentations in patients with HBV and / or HCV infection

	Normal	Mild	Medium	Severe	Fatty liver	Not detected	Total
Group BC	4	0	2	1	3	30	10
Group B	8	0	2	0	3	3	13
Group C	0	13	14	1	2	1	31

Clinical outcome of patients with HBV and HCV coinfection

All the patients had no apparent symptoms and physical signs of liver diseases. In Group BC, 10 of them (25 %) had abnormal liver enzyme values, and the highest value of ALT was 218 u/L with a mean of 95±72 (u/L) (normal range for ALT is 0-40 u/L). Five patients in Group B and 12 in Group C had elevated liver enzyme values. The rate of abnormal biochemical values was similar among the groups. The mean of elevated ALT values was 69±33 (u/L) in Group B and 53±16 (u/L) in Group C. Fifty three of the patients underwent B type ultrasound detection, and most of them had mild or moderate abnormal ultrasonic manifestations according to the criteria formulated by Chinese Medical Association[24] (Table 5). Other liver enzyme values such as TBIL, ALP, GGT and AFP were in the normal range in all the patients, and no one was found to have HCC.

DISCUSSION

All the patients were individual blood donors with chronic HBV and/or HCV infection. They have never received interferon or other antiviral agent treatment, thus our data partly reflected the natural history of HBV and HCV coinfection.

High clearance rate of both viruses, and mild clinical manifestations of coinfection were the prominent findings in this study. It may have some relationship with the special characteristics of the patients. First, different from those who got HBV and HCV coinfection sporadically, the patients in our study were all individual blood donors, and had a history of taking plasma from the blood and transfusion back of the blood cells. Second, they were adults when they acquired the infection. During 1982 to 1989, they provided the blood 5-15 times, and 200 to 400 ml each time. Third, they were the natives of certain villages in Hebei Province and their blood was collected in the same hospitals. These characteristics of the patients may have some relationships with the high clearance rate of HBV in Group BC and Group B. The mechanism needs to be further studied. After 14 to 21 years of HBV and HCV coinfection, 87.5 % of the patients have cleared HCV RNA. Researchers in Western countries reported that the spontaneous clearance rate of HCV RNA was less than 15 % in adults with HCV infection, and 34 % in patients with coinfection[25]. The history of HCV infection in China was different from that in Western countries, 29 % of the patients who got HCV infection after blood transfusion for 12 to 25 years have cleared the virus, and the clinical manifestations were occult[21]. This may be the reason of different characteristics of patients with coinfection in China from those in Western countries.

With regard to the relationship of HBV/HCV coinfection and clinical outcome, most researchers found that coinfection of HBV and HCV could cause more severe liver damage than single infection[2,13-16]. While in this study, neither patients with coinfection nor patients with single HBV or HCV infection had obvious symptoms and signs of liver diseases. Twenty five percent of patients with coinfection had elevated liver enzyme values. The mean of elevated ALT values was 95±72 u/L, while other liver enzyme values such as TBIL, ALP, and GGT were in the normal range. In most patients the ultrasonic presentations changed mildly. Good clinical outcome was one of the main characteristics of the patients with coinfection in this study. It was reported that in patients with HBV and HCV coinfection after orthotopic liver transplantation, the presence of HCV might improve the clinical outcome as compared with HBV infection alone[20]. Utili *et al*[25] followed up a group of cancer survival children who acquired HBV and HCV during treatment of neoplasia for a median period of 13 years, in which the patients went though a chronic indolent course of the liver disease, 59 % of them lost one or both viruses over time.

The clearance rate of HCV RNA in Group BC was 87.5 % in 2002, significantly higher than that in Group C (32.3 %) (P<0.05), and Group BC had a higher clearance rate of HCV RNA compared with Group C (P<0.05) (Table 3) in 1994. It was suggested that in HBV and HCV coinfection group the two viruses had mutual interference, and HBV suppressed HCV replication. While in Group BC, the patients who were positive for HCV RNA in 1994 were negative for HBV DNA in 2002, and vice versa. Previous studies also found that HBV could inhibit HCV replication and took the leading role in chronic hepatitis[9-12]. Acute HBV superinfection of patients with chronic HCV infection could suppress their pre-existing HCV, and the timing or sequence of infection was a factor influencing the outcome of viral interactions[22, 26]. The mechanism might

be that antiviral cytokines such as IFN-γ and TNF-α produced by non-T cells in the event of superinfection could inhibit the pre-existing virus[27]. *In vitro* experiments showed that when HBV DNA and HCV RNA were co-transfected into HuH7 cells, HBV DNA suppressed HCV RNA secretion, and HCV RNA also suppressed HBsAg secretion in comparison with either of HCV RNA or HBV DNA transfection alone[28].

Former studies showed that HCV could inhibit HBV replication[5-8, 29], but in this study, no statistical difference was found in the clearance rate of HBsAg, HBeAg and HBV DNA between Group BC and Group B. We did not find any relationship between serum HCV RNA and HBV DNA levels in patients with coinfection. The small number of patients in Group B might result in statistical discrepancy. Anyway, no evidence was found in this study to support that HCV could affect HBV replication.

Considering that China is a highly endemic area of HBV, and most of patients with chronic HBV infection acquired the virus in their infancy, our data may partly reflect the natural history of HBV infection in adults. Its characteristics are as follows.

High clearance rate of HBV and indolent course of the infection After 14 to 21 years of infection, the clearance rates of HBsAg, HBeAg and HBV DNA in Group BC were 67.5 %, 92.5 % and 87.5 %, while in Group B, the clearance rates of these markers were 50 %, 87.5 % and 81.5 % respectively, and no significant difference was found between the two groups. It was reported that 5-10 % of chronically infected patients cleared HBV DNA and HBeAg spontaneously each year, and this might be followed by clearance of HBsAg[30,31]. In Taiwan the annual clearance rate of HBsAg was 0.43 %[32]. European Association for the Study of the Liver (EASL) reported that in Western countries, about 1-2 % of HBV carriers became HBsAg negative each year, while in endemic areas the rate of HBsAg clearance was lower (0.05-0.08 % per year)[23]. In chronic coinfection the clearance rate of HBsAg seemed higher than that in single HBV infection, and 2.03 % of patients in Taiwan cleared HBsAg annually[32]. In this study, either in patients with HBV/HCV coinfection or in HBV single infection, the clearance rate of HBsAg was higher than that ever reported. And they had mild clinical manifestations and no evidence to progress to more severe diseases.

It seemed easier for man to eliminate HBV We found in Group BC, most of the males (85.7 %) cleared HBV, and only 47.4 % of females did so ($P<0.05$). In Group B, no statistical difference was found, which might be due to a small number of cases. It was also reported in a long-term follow-up study that 88.9 % of the patients who cleared HBsAg were males[32].

The virus load did not influence the clearance rate of HBV There is no statistically significant difference of clearance rate of HBV between different virus load groups.

In short, mild and occult clinical manifestations and high clearance rate of both viruses were the characteristics of patients with HBV/HCV coinfection. High clearance rate of HBV in male patients was a clinical feature in adults with HBV infection. HBV could inhibit HCV replication, but no evidence was found that HCV could suppress HBV replication.

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Edited by Zhang JZ and Wang XL