

Prospective study in 142 cases of hepatitis C virus infection

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Abstract

AIM: There is limited information on the natural history of HCV infection in China. We investigated the outcome of HCV infection after nine-year follow-up and the risk factors in blood donors in China in order to provide the foundation for prevention and therapy.

METHODS: A total of 172 cases of HCV infection with anti-HCV positive and ALT abnormality were enrolled in the archives when was screened blood in Hebei Province in 1993. In them 142 blood donors were followed up till July 2002. No antiviral treatment was applied to them during the period of infection. In the present study, anti-HCV, HCV-RNA and aminotransferase were detected and genotyping was conducted by the method of restriction fragment length polymorphism(RFLP). B-type ultrasound detection was performed in all the patients. Age, sex, alcohol consumption and clinical symptoms were questioned.

RESULTS: After nine years' follow-up, 10.56% (15/142) of the cases were negative for anti-HCV and 16.42% (12/134) of them were negative for HCV-RNA. The genotypes 1b, 2a and 1b/2a were 91.07%, 6.25% and 2.68% respectively. Twelve cases (8.45%) were negative for both HCV RNA and anti-HCV. The rate of chronicity in this group was 83.58% (112/134), and the rate of viral spontaneous resolution was 16.42% (22/134). The mean level of ALT, AST, γ -GT in HCV RNA positive cases was significantly higher than that in HCV RNA negative cases ($P < 0.001$). The abnormal rate of ALT and/or AST in male donors was significantly higher than that in female donors ($P = 0.005$). The rate of progression to liver cirrhosis from chronic hepatitis C was significantly higher in the cases of super-infection with HBV than that in the cases of single HCV infection. Overdose alcohol consumption promoted the progression to chronicity.

CONCLUSION: This area (Hebei Province) has a higher rate of chronicity in HCV infection, and measures should be taken to prevent its progression to serious liver diseases, especially for patients super-infected with HCV and HBV.

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INTRODUCTION

Hepatitis C virus is the major cause of post-transfusion hepatitis. It has been estimated that nearly 170 million people are infected with HCV in the world. High chronicity is an obvious characteristic of HCV infection. Eighty-five percent of infected patients developed chronic infection and 8-45% of them could resolve. Liver cirrhosis developed in 10-50%, hepatocellular carcinoma in 1-23%, liver disease related mortality in 4-15%^[1-3] in twenty years of natural infection. There is limited information about the long-term follow-up study in China. Many donors were infected with HCV by means of plasmapheresis in the 1990 s^[4]. In the present study, 172 cases of blood donors infected with HCV were diagnosed in August of 1993, and 142 of them were followed up till July 2002. The outcome in natural history of HCV infection and related factors affecting the outcome were investigated.

MATERIALS AND METHODS

Patients

A total of 172 cases of blood donors from a rural area of Zhao County in Hebei Province were diagnosed as HCV infection by etiology and biochemistry detection (including anti-HCV and hepatitis B surface antigen) in August of 1993. Thirteen cases of them were co-infected with hepatitis C virus and hepatitis B virus. Nine years later, only 142 persons (66 men and 76 women with a mean age of 36.67±9.10 years at time of infection and 45.70±9.11 years at time of investigation) were followed up. Additionally, three children with aged of 2 to 8 years at the time of infection were checked-up in our series. It was noted that all of the donors received no antiviral treatment during the nine years of infection.

Methods

Anti-HCV antibodies Five-milliliter peripheral vein blood was collected for biochemical and etiology detection. All patients were tested for the presence of anti-HCV antibodies with Abbott diagnostic test kit (Abbott, USA) according to the manufacturer's instructions in 1993 and 2002. The rest of the blood samples were stored at -80 °C.

HCV RNA detection and genotyping Primers were designed from the conserved 5'-noncoding (5'-NC) region of the HCV genome. P1: 5'-GTGTGAGGAAGTACTGTGTT-3', P1': 5'-AACACTACTCGGCTAGCAGT-3', and P2: 5'-TTCACGCAGAAAGCGTCTAG-3', P2': 5'-GTTTATCAAGAAAGGACCCG-3'. RT-PCR procedure was performed as previously described^[5]. PCR products positive for HCV RNA were genotyped with restriction fragment length polymorphism (RFLP) method^[5].

Risk factors for HCV chronicity All data of risk factors for HCV chronicity were collected by using special questionnaires, including sex, age, symptoms, alcohol consumption, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyltransferase (γ -GT), alkaline phosphatase (ALP), total bilirubin (Tbil), direct bilirubin (Dbil), total protein (TP) and

albumin (ALB) in 12 by using fully automatic biochemical analysis compliance 7071A (Hitachi, Japan). HBV markers were also detected by Abbott diagnostic kit.

Diagnostic criterion Chronic hepatitis C and liver cirrhosis were diagnosed based on the criteria modified at the Sixth (Xi'an) National Convention of Infectious, Parasitic and Liver Diseases^[6].

Statistical analysis

Quantitative values were expressed as mean±SE. Unpaired Student's *t* test, χ^2 and Fisher's exact test were used for statistical comparison of the data. $P < 0.05$ was considered statistically significant.

RESULTS

Outcome of HCV infection

In the 172 cases enrolled in this study in 1993, seven cases were already died at the time of investigation. Two (1.16%) out of seven cases died of suspected hepatocellular carcinoma. Twenty-three cases were out of follow-up. In the present study, only 142 cases were followed-up under the investigation. Out of the 142 cases, HCV RNA was detected in 134 cases. The result showed that 112 cases (83.58%) of the donors were positive for HCV RNA and 16.42% were negative for HCV RNA; 89.44% were positive for anti-HCV and 10.56% were negative for anti-HCV; 8.45% of them were negative for both anti-HCV and HCV RNA. Out of the 142 infected persons, most but one (who was with chronic moderate hepatitis C) with mild chronic hepatitis C had no obvious clinical symptoms according to the viral hepatitis prevention project. In the cases with persistent infection, B-type ultrasound detection showed 2.68% with liver cirrhosis, 86.61% with chronic hepatitis and 10.71% with fatty liver.

Anti-HCV, HCV RNA and abnormality of liver function

Ninety-one point sixty percent of the cases were positive for HCV RNA in the positive group of anti-HCV. Eight cases of severe hepatitis and three cases of liver cirrhosis were positive for both anti-HCV and HCV RNA. Another two patients with liver cirrhosis and severe hepatitis were co-infected with both HCV and HBV in 1993 and turned positive for anti-HCV, negative for HCV RNA and positive for hepatitis B surface antigen in the present study. The rate of ALT and/or AST abnormalities in the positive group of anti-HCV was 28.57% (34/119), while that in the negative group of anti-HCV was zero (Table 1). The average level of ALT, AST and γ -GT in the positive group of HCV RNA was significantly higher than that in the negative group ($P < 0.001$) (Table 2). On the other hand, the abnormal rate of ALT and / or AST in the positive group was also significantly higher than that in negative group (29.46% vs 4.55%, $P = 0.014$).

Clinical symptoms

Fifty out of 112 cases (44.64%) of chronic HCV infection had mild symptoms, including abdominal pain (18.75%), fatigue (17.86%), and arthralgia (16.96%). The rates of cases with symptoms in the group of liver cirrhosis was 50.0%. Surprisingly, six cases with negative viral markers had fatigue.

Table 1 Abnormal of serum alanine aminotransferase in patients infected with HCV after 9 years' follow-up in different groups

Group	Liver function	
	Normal cases (%)	Abnormal cases (%)
Anti-HCV(+)/HCV RNA(+)	76 (69.72)	33 (30.28)
Anti-HCV(+)/HCV RNA(-)	9 (90.00)	1 (10.00)
Anti-HCV(-)/HCV RNA(+)	3 (100.0)	0
Anti-HCV(-)/HCV RNA(-)	12 (100.0)	0
Anti-HCV(+)/HCV RNA(undetected)	4 (50.00)	4 (50.00)
Total	104 (73.24)	38 (26.76)

Note: The number in the bracket is the percentage of normal and abnormal cases in each group.

Co-infection with HBV and alcohol consumption

Thirteen of 142 cases had co-infection with HBV in 1993. Twelve cases of them were positive for hepatitis B surface antigen (HBsAg) and the rest were negative for HBsAg and positive for anti-hepatitis B core (anti-HBc). Nine years later, three of them turned negative for HbsAg, suggesting that spontaneous resolution of HBsAg per year was 2.77% similar to the of standard rate of resolution (2.01%) in our country^[7]. The rate of spontaneous resolution of anti-HCV was 7.69% in the group of co-infection with HBV while 8.53% in the group of single HCV infection. There was no significant difference between the two groups. Thirty point seventy-seven percent lost their HCV RNA in the group super-infected with HCV and HBV and 14.88% lost their HCV RNA in the group with single HCV infection. However, there was no significant difference between them. Two (15.38%) out of thirteen cases with co-infection developed liver cirrhosis while two (1.55%) out of 129 cases with single HCV infection also developed liver cirrhosis. The progression to liver cirrhosis due to co-infection was significant faster than that due to single infection ($P < 0.05$). Thirteen of 142 cases had alcohol consumption of more than 100 g every day and all developed persistent infection. We also found that all cases with viral resolution had no history of excessive alcohol consumption, suggesting that alcohol consumption might promote the progression of HCV infection to chronicity.

Age at the time of primary infection

We divided the donors into three groups according to the age of infection: 18-30 years, 31-40 years and ≥ 41 years. The rate of spontaneous viral resolution in the three groups was 17.95%, 15.09% and 16.67% respectively. There was no significant difference. There was no linear correlation between the age of infection and the viral load ($r = -0.144$). Liver function was normal in the three investigated children. One of them was negative for viral markers and the other two developed persistent infection.

Influence of sex on the viral resolution

Sixty-two male cases and seventy-two female cases were detected for HCV RNA. The rate of viral resolution was 12.90% in the male group and 19.44% in the female group. No significant

Table 2 Comparison of ALT, AST, ALP, γ -GT, TBIL and DBIL between positive (+) and negative (-) HCV RNA patients (mean±SE)

HCV RNA	Case	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	γ -GT (IU/L)	Tbil (μ mol/L)	DBil (μ mol/L)
+	112	31.2±32.6 ^b	34.8±27.0 ^b	82.0±36.6	20.0±17.6 ^b	13.2±5.6	6.0±2.3
-	22	16.1±8.6	22.9±6.5	78.9±40.1	12.4±5.8	12.1±7.4	5.4±2.2

^b $P < 0.001$ vs the negative group of HCV RNA.

difference was found between them. Four cases of liver cirrhosis were all male cases. The rate of ALT and/or AST abnormalities was significantly higher in male donors than in female donors ($P=0.005$).

Related factors affecting the outcome of HCV might include co-infection with HBV and long-term excessive alcohol consumption, which could promote the progression of HCV infection to chronicity and liver cirrhosis. In the present study, we could not find the significant correlation between the outcome of HCV and age of infection and sex.

DISCUSSION

The harm of hepatitis C is only next to hepatitis B in our country. The epidemic rate is 3.2% in Chinese population and it is estimated that 41 million people were infected^[8]. Economic burden for HCV is 11.7-21.6 billion RMB per year in China. New cases have been decreased through the detection of blood donors since 1990 s, but part of patients would progress to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Although there are many reports on this aspect in Western countries^[9-13], few similar researches have been carried out in China. So it is very necessary to summarize the natural outcome of HCV in China.

Chronicity rate

The rate of spontaneous resolution in the present study was 16.42%, lower than 29.01%^[14] reported before in China after 12-25 years of infection. It may be because the qualitative method used in the study was more sensitive than quantitative method used in another area. Additionally, during our investigation, the infection was spontaneously resolved in some cases, which might be one of the causes of high chronicity in HCV infection in this study. Two patients died of suspected liver disease, and the case fatality rate was 1.16% in the group studied, which was lower than the previously reported^[10]. B-type ultrasound detection reflected liver inflammation in some degree under the condition that liver biopsy was not taken. After nine years of infection, 2.68% of the patients developed liver cirrhosis, which was lower than 7-16% after 8-16 years of infection^[1]. Our results showed that the clinical symptoms were latent in the donors studied. Compared with the patients with HBV infection, higher chronicity and faster progression to liver cirrhosis were observed in the patients with HCV infection.

Factors affecting the outcome

Age As described previously^[15-17], age appear to be an important determinant of progression. The data suggest that the younger the age at infection, the lower the rate of progression. In the present study, no difference was found in the rate of viral resolution in the different age groups, which is consistent with a previous report^[14]. The rate of chronicity was higher in the group than in children and young women of other countries.

Sex Regarding sex, there is evidence that the rate of progression of liver disease was lower in women than in men. The cases who progressed to liver cirrhosis in our study were all males. The rate of ALT and/or AST abnormalities in males was significantly higher than that in females, indicating that males might relate with the more serious liver disease.

Super-infection with HBV The rate of viral resolution in the donors super-infected with HBV was a little higher than in the donors without HBV infection, but there was no significant difference. The rate of progression to liver cirrhosis in the cases super-infected with HCV and HBV was significantly higher than that in the cases only infected with HCV, which is consistent with previous reports.

Alcohol consumption Several reports showed that overdose alcohol consumption might contribute to the progression to liver cirrhosis and hepatocellular carcinoma. In the present study, alcohol consumption was shown the progression to chronicity. Whether alcohol consumption affects the progression of liver diseases, needs to be further studied.

In summary, this area (Hebei province) has a higher rate of chronicity in HCV infection, and measures should be taken to prevent the progression to serious liver diseases, especially for the patients super-infected by HCV and HBV.

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