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Prevalence of Antibodies to Hepatitis C Virus in Patients with Various Types of Liver Diseases

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Background: *Hepatitis C virus (HCV) is known to be a major cause of non-A, non-B hepatitis (NANBH) and is thought to be an important causative agent of serious liver disease. Recently the role of HCV in the development of various liver disease is suggested.*

Methods: *Sera from 222 patients with various liver diseases had been kept frozen at -20 °C until the test. Anti-HCV was detected using the ABBOTT HCV EIA Test System (ABBOTT Co., America) following the manufacturer's instructions. The assay uses a recombinant HCV antigen (C 100-3) synthesized in yeast.*

Results: *HCV antibodies (anti-HCV) were detected in 35 (31.5%) of 111 HBsAg-negative patients. The prevalence rate of anti-HCV was 61.9% (13 out of 21 patients) in chronic hepatitis, 29.1% (14 out of 48) in liver cirrhosis, 26.3% (5 out of 19) in hepatocellular carcinoma and 13% (3 out of 23) in acute hepatitis was far less (3 out of 111 patients, 2.7%) than that of HBsAg-negative patients ($p<0.01$). In this group, anti-HCV was detected in 2 (5.1%) out of 39 liver cirrhosis, 1 (1.9%) out of 52 chronic hepatitis, among them 47 were biopsy-proven chronic active hepatitis, and none of 20 hepatocellular carcinoma.*

Conclusions: *These data suggest that, in Korea, 1) coinfection of HCV and HBV is infrequent, 2) HCV might be an important cause of HBsAg-negative chronic hepatitis, 3) HCV is seemed to be a less likely important factor associated with liver cirrhosis or hepatocellular carcinoma in HBsAg-negative patients, but further prospective study with a large population is necessary.*

Key Words: *Non-A, non-B Hepatitis (NANBH), Hepatitis C virus (HCV), anti-HCV*

INTRODUCTION

Since the development of sensitive diagnostic tests for infection of hepatitis A virus (HAV) and hepatitis B virus (HBV) in 1975, it was well known that most cases of post-transfusion hepatitis are not caused by these agents or any other known hepatotropic virus such as cytomegalovirus (CMV) or Epstein-Barr virus (EBV)[1]. Although most acute infections are asymptomatic, at least half of these non-A, non-B (NANB) infections result in chronic hepatitis, which may result in cirrhosis in approximately 20% of cases[2]. A potential association with hepatocellular carcinoma has also been proposed[3,4]. In addition, NANB virus is a frequent cause of community-acquired (sporadic) hepatitis, a nonpercutaneously transmitted hepatitis that is also often chronic[5]. Despite intensive research over a decade, the causative agent (or agents) of these non-A, non-B hepatitis (NANBH) remained unidentified. However, in the late 1970s, transmission of the agent to chimpanzees was reported[6,7]. As in humans, about 50% of infected chimpanzees develop chronic NANB infections following inoculation with contaminated human serum or

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blood clotting concentrates[8]. These chimpanzee transmission studies have defined a NANB agent isolated form clotting factor VIII concentrates, which is chloroform sensitive, and induces ultrastructural cytoplasmic tubular changes in hepatocytes. A system for the detection of the non-A, non-B virus has been elusive, but such a system has been reported since the recent cloning of the genome of the previously uncharacterized NANB virus[9], now tentatively designated the hepatitis C virus; both radioimmune and enzyme-linked assays have been developed to detect antibody (anti-HCV) to the protein expressed in the cloning experiments[5]. We tested anti-HCV, using the enzyme-immunoassay, in serum samples from patients with various liver diseases to assess the role of HCV infection in the development of liver diseases in Korea.

SUBJECTS AND METHODS

1. Patients

222 patients with various types of liver diseases, diagnosed between 1987 and 1991, were studied. Sera from these patients had been kept frozen at -20°C until the test. Of these, serum HBsAg-negative patients were 111 and the remainder were positive for HBsAg. HBsAg-negative group consisted of 23 patients (M=14, F=9) with acute hepatitis who were negative for serum HBsAg, HBeAg and anti-HAV (IgM), 21 with chronic hepatitis, of whom 9 (M=6, F=3) were biopsy-proven chronic active hepatitis, 48 (M=37, F=11) with liver cirrhosis and 19 (M=15, F=4) with hepatocellular carcinoma. In HBsAg positive group, there were 52 patients (M=36, F=16) with chronic hepatitis consisting of 47 with biopsy-proven chronic active hepatitis, 39 (M=29, F=10) with liver cirrhosis and 20 (M=15, F=5) with hepatocellular carcinoma. Diagnosis of non-biopsy proven chronic hepatitis was made when the elevated serum alanine aminotransferase (sGPT) persisted at least 6 months. Liver cirrhosis was diagnosed based upon abnormal blood chemistry and physical findings and presence of any evidence of portal hypertension either on radiologic or endoscopic examinations. Diagnosis of hepatocellular carcinoma was confirmed either by histologically, radiologically (such as ultrasonography, computed tomography, radionuclide scanning or angiography of the liver) or significantly elevated serum alpha-fetoprotein (AFP) concentration.

2. Serologic Tests

1) Markers for Hepatitis A and Hepatitis B

Anti-HAV was tested with radio-immunoassay using HAVAB-M® (ABBOTT Co., America). HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc were tested with a commercially available enzyme immunoassay using AUSZYME®, HBe (rDNA) EIA, AUSAB®, CORZYME® tests, respectively (ABBOTT Co., America).

2) Antibody to Hepatitis C Virus (anti-HCV)

Anti-HCV was detected using the ABBOTT HCV EIA Test System (ABBOTT Co., America) following the manufacturer's instructions. The assay uses a recombinant HCV antigen (C 100-3) synthesized in yeast. The presence or absence of antibody to HCV is determined by relating the absorbance of the specimen to the cutoff value. The cutoff value is the mean absorbance of the negative control plus 0.25 times the mean absorbance of the positive control. For the run to be valid, the difference between the mean absorbances of the positive and negative controls (P-N) should be 0.400 or greater. Specimens with absorbance values less than the cutoff value are considered negative and specimens with absorbance values greater than or equal to the cutoff value are considered initially reactive, but, before interpretation, the original sample should be retested in duplicate. If either duplicate retest is reactive, the specimen is interpreted to be repeatably reactive for antibodies, but initially reactive specimens which do not react in both of the duplicate tests are considered negative by the criteria of ABBOTT HCV EIA.

STATISTICAL ANALYSIS

Statistical significance was determined using the binomial test. A p value$\leq$0.05 was considered significant.

RESULTS

1) Prevalence of anti-HCV in HBsAg-negative patients with various liver diseases.

Serum anti-HCV was detected in 7 (77.7%) among 9 biopsy-proven CAH patients, 13 (61.9%) among 21 patients with chronic hepatitis, 14 (29.1%) among 48 patients with liver cirrhosis, 5 (26.3%) among 19 patients with hepatocellular carcinoma and 3 (13%) among 23 patients with acute hepatitis (Table 1)

2) Prevalence of anti-HCV in HBsAg-positive

Table 1. Prevalence of Anti-HCV in HBsAg-negative Patients with Various Liver Diseases

Diagnosis	No. patients	No. positive	% positive
Acute Hepatitis	23	3	13
Chronic Hepatitis	21	13	61.9
*(CAH)	(9)	(7)	(77.7)
Liver Cirrhosis	48	14	29.1
Hepatocellular Carcinoma	19	5	26.3
Total	111	35	31.5

*CAH=Biopsy-proven chronic active hepatits

Table 2. Prevalence of Anti-HCV in HBsAg-positive Patients with Chronic Liver Diseases

Diagnosis	No. patients	No. positive	% positive
Chronic Hepatitis	52	1	1.9
(CAH)	(47)	(0)	(0)
Liver Cirrhosis	39	2	5.1
Hepatocellular Carcinoma	20	0	0
Total	111	3	2.7

Table 3. Relationship between Anti-HCV Prevalence and the Appearance of Other Hepatitis B Viral Markers (anti-HBs and anti-HBc) in HBsAg-negative Chronic Liver Diseases

anti-HBc/anti-HBs	No. patients	No. positive (%)
+/+	41	12(29.2)
+/−	21	8(39.0)
−/−	12	5(41.6)
−/+	5	3(60.0)

patients with chronic liver diseases.

Serum anti-HCV was detected in 2 (5.1%) among 39 patients with liver cirrhosis, one (1.9%) among 52 patients with chronic hepatitis, none among 47 biopsy-proven CAH patients and none among 20 patients with hepatocellular carcinoma (Table 2).

3) Relationship between the appearance of other HBV markers and the prevalence of anti-HCV in the HBsAg-negative chronic liver diseases.

Serum anti-HCV was detected in 3 (60%) among 5 patients positive for anti-HBs only, 5 (41.6%) among 12 patients negative for anti-HBs and anti-HBc, 8 (38.0%) among 21 patients positive for anti-HBc only and 12 (29.2%) among 41 patients positive for anti-HBs and anti-HBc (Table 3).

4) Difference in the prevalence of anti-HCV between various HBsAg-negative chronic liver diseases.

The prevalence of anti-HCV in patients with chronic hepatitis was significantly higher than the prevalence in patients with liver cirrhosis ($p<0.01$) and primary liver cancer ($p<0.05$). There was no significant difference between liver cirrhosis and hepatocellular carcinoma.

DISCUSSION

We tested anti-HCV in sera from patients with various liver diseases to define the role of HCV infection in liver disease in Korea. The results indicate that HCV is a major cause of HBsAg-negative chronic liver disease, especially chronic hepatitis. The overall prevalence of anti-HCV in patients with HBsAg-negative chronic hepatitis was 61.9%. Similar results were reported in foreign countries (60~70%[5,10]), but the results in Korea ranged from 15% to 56.7%[11~13]). The prevalence of anti-HCV in HBsAg-negative patients with liver cirrhosis (29.1%) and primary liver cancer (26.3%) was markedly lower than the reports from foreign countries. Other reports from Korea also indicate the similar lower prevalences (15~40%) in patients with liver cirrhosis and primary liver cancer[11~15]. The lower prevalence of anti-HCV in liver cirrhosis and hepatocellular carcinoma than in chronic hepatitis is difficult to explain at present. Although HCV infection is seemed to be the most frequent cause of non-B chronic hepatitis in Korea, progression to cirrhosis and cancer may be also associated with other etiologic agents, such as alcohol, drugs and aflatoxins. The low prevalence of anti-HCV in non-B acute hepatitis (13%) may be due to the lower titer and unstable structure of the antibodies formed during the acute phase[17] and delayed appearance of the antibodies in the acute infection of HCV[5,17].

We tested serum anti-HCV in HBsAg-positive patients with chronic liver diseases to evaluate the role of HCV coinfection in the progression of chronic liver diseases in HBV carriers. There was a report of increased prevalence of anti-HCV according to the severity of chronic liver diseases in HBV carriers in Italy[4], suggesting that the concerted action of dual HBV and HCV infection may enhance the progression of chronic liver diseases and hepatic carcinogenesis. However, the prevalence of anti-HCV in HBsAg-positive patients with chronic liver diseases was low (2.7%) and not

related to the severity of chronic liver diseases. The similar results of low prevalence of anti-HCV in HBsAg-positive patients were shown in other reports from Korea[12,15,16]. It may be partially due to the difference in the mode of transmission between HBV and HCV. In Korea, a majority of chronic persistent HBV infections appear to be caused by perinatal vertical transmission from HBV carrier mothers, whereas there is no definite evidence for vertical transmission of HCV.

In conclusion, coinfection of HBV and HCV is uncommon and HCV infection might be an important cause of non-B chronic hepatitis in Korea. The role of HCV infection in the development of liver cirrhosis and hepatocellular carcinoma in HBsAg-negative patients appears to be minor in Korea, but further studies are required to define it clearly.

REFERENCES

1. Feinstone SM, Kapikian AZ, Purcell RH, Alter HJ, Holland PV: *Transfusion-associated hepatitis not due to viral hepatitis type A or B. N Engl J Med 292:767, 1975*
2. Dienstag JL, Alter HJ: *Non-A, non-B hepatitis. Evolving epidemiologic and clinical perspective. Seminars in Liver Disease 6:67, 1986*
3. Bruix J, Barrera JM, Calvet X, Ercilla G, Costa J, Sanchez-Tapias JM, Ventura M, Vall M, Bruguera M, Bur C, Castillo R, Rodes J: *Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet ii:1004, 1989*
4. Colombo M, Kuo G, Choo Q-L, Donato MF, Ninno ED, Tommasini MA, Dioguardi N, Houghton M: *Prevalence of antibodies to hepatitis C virus in Italian patients with primary liver cancer. Lancet ii:1006, 1989*
5. Kuo G, Choo Q-L, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, miyamura T, Dienstg JL, Alter MJ, Stevens CE, Tegtmeier GE, Bonino F, Colombo M, Lee WS, Kuo C, Berger K, Shuster JR, Overby LR, Bradley DW, Houghton M: *An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 244:362, 1989*
6. Taber E, Gerety RJ, Drudker JA, Seeff LB, Hoofnagle JH, jackson DR, April M, Barker LF, Tamondong GP: *Transmission of non-A, non-B hepatitis from man to chimpanzee. Lancet 1:463, 1978*
7. Alter HJ, Purcell RH, Holland PV, Popper H: *Transmissible agent in non-A, non-B hepatitis. Lancet 1:459, 1978*
8. Bradley DW, Maynard JE, Popper H, Ebert JW, Cook EH, Fields HA, Kemler BJ: *Persistent non-A, non-B hepatitis in experimentally infected chimpanzees: J Infect Dis 143:210, 1981*
9. Choo QL, Kuo G, Weimer AJ, Overby LR, Bradley DW, Houghton M: *Isolation of cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244:359, 1989*
10. Roggendorf M, Deinhardt F, Rasshofer R, Eberle J, Hopf U, Moller B, Zachoval R, Pape G, Schramm W, Rommel F: *Antibodies to hepatitis C virus. Lancet 11:324, 1989*
11. Shim Sang-Koon, Lee Jae-Koo, Choi Jin-Hyun, Jo Choong-Hyun, Kim Young-Jae, Choi Jin-Hak: *A study on the detection rate of hepatitis C virus in patients of various kinds of liver disease. Abstract of the Korean Journal of Internal Medicine, 1990*
12. Park Young-Min, Han Nam-Ik, Kim In-Shik, Im Kye-Soon, Jung Jin-Woo, Lee Chang-Dong, Kim Boo-Sung: *The frequency of anti-HCV appearance in various kinds of liver disease. Abstract of the Korean Journal of Internl Medicine, 1990*
13. Jeong Kyoo-Won, Sun Heu-Shik, Jung Hwan-Keuk, Shin Ho-Koon, Park Choong-Ki, You Jae-Young: *The infection of hepatitis C virus in patients of posttransfusion hepatitis and chronic liver disease in Korean 9th first step). The Korean Journal of Int. Med. 38:750, 1990*
14. Jeong Mi-Ryoung, Kim Kyoung-Heui, Lee Kwan-Shik, Han Kwang-Kyb, Jun Jae-Yoon, Lee Sang-In, Moon Young-Myoung, Kang Jin-Kyoung, Park In-Seo, Choi Hong-Jae: *A study on the detection of anti-HCV and HBV markers in the serum in patients of liver cirrhois and liver cell cancer. Abstract of the Korean Journal of Int. Med., 1990*
15. Jo Sung-Won, Hwang Sung-Kyoo, Jo Joo-Young, Lee Moon-Sung, Kim Jin-Hong, Shim Chan-Seob: *The prevalence of hepatitis C virus in the liver cirrhosis and hepatocellular carcinoma. Abstract of the Korean Journal of Internal Medicine, 1990*
16. Hwang I-Sook, Kim Hyo-Jong, Kim Byoung-Hyo, Lee Jung-Il, Jang Young-Woon, Jang Rin: *The infection mode of the hepatitis C virus in patients of HBsAg-positive and HBsAg-negative chronic liver diseases. Abstract of the Korean Journal of Internal Medicine, 1990*
17. Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M, Kuo G: *Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Eng J Med 321:1494, 1989*