SEROPREVALENCE OF ANTIBODIES TO THE HEPATITIS C VIRUS IN SINGAPORE

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Abstract. The prevalence of antibodies to the hepatitis C virus (anti-HCV) in Singapore was assessed using a recombinant-based enzyme linked immunoassay system. 1004 serum samples were obtained from normal subjects (463), hemodialysis patients (112), hepatitis B virus (HBV) carriers (188), patients with hepatocellular carcinoma (HCC) (58) and patients with non-hepatitis B virus related liver diseases (183). Anti-HCV was found to be positive in 1.7% of healthy subjects, and in 20% of patients on regular hemodialysis. Three percent of HBV carriers were positive for anti-HCV. Twelve percent of patients with acute hepatitis with no known causes and 20% patients with chronic hepatitis with no known causes were positive for anti-HCV. Among patients with cirrhosis for which no known causes were found 33% were positive for anti-HCV. Thirty six percent of patients with HCC not associated with the presence of HBsAg were positive of anti-HCV. None of the patients with known causes of liver disease were positive for anti-HCV.

INTRODUCTION

Post-transfusion hepatitis caused by an agent other than hepatitis B was first recognized in 1974 (Prince *et al,*1974, Feinstone *et al,* 1975). More than 90% of transfusion associated hepatitis cases worldwide are attributable to non-A non-B hepatitis (NANBH) (Feinstone *et al,* 1975) which also accounts for a substantial proportion of hepatitis cases among patients with frequent parenteral exposure to blood [eg hemophiliacs (Fletcher *et al,* 1983), intravenous drug abusers (Mosley *et al,* 1977) and hemodialysis patients (Galbraith *et al,* 1975)]. Non-A non-B hepatitis is also responsible for more than 25% of cases of sporadic hepatitis without obvious percutaneous exposure worldwide (Alter *et al,* 1982; Francis *et al,* 1984). A blood borne NANBH agent has recently been isolated and designated the hepatitis C virus (HCV) (Choo *et al,* 1989). Virus isolation led to the development of a recombinant-based immunoassay for detection of specific anti-HCV antibodies (Kuo *et al,* 1989). We studied sera from 1,004 individuals to estimate the frequency of HCV infection in Singapore.

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MATERIALS AND METHODS

Sera from the following groups of subjects were studied. None of the patients were known intravenous drug abusers and none of the males were homosexuals.

Healthy subjects

Four hundred and sixty-three healthy volunteers who had enrolled in HBV vaccination trials between 1988 and 1989 were studied. None of them had a history of transfusion of blood or blood products.

Hemodialysis patients

One hundred and twelve patients with chronic renal failure on maintenance hemodialysis at four centers [Tan Tock Seng Hospital, Alexandra Hospital, Singapore General Hospital (67 patients) and National University Hospital (45 patients)] were studied.

Patients with liver diseases.

Four hundred and twenty-nine patients with

various liver diseases including

188 consecutive chronic hepatitis B carriers seen by two of the authors (RG and IY) at the specialist outpatient clinic, National University Hospital over a period of one month.

58 patients with hepatocellular carcinoma proven by any three of the following : abdominal ultrasound, computerized tomography, histology, and raised alpha-fetoprotein levels.

183 patients with HBsAg negative liver diseases. Thirty-four patients had acute hepatitis not due to the presently known hepatotropic viruses in whom drug hepatitis has been excluded from the history. The rest had chronic liver disease : 55 had chronic hepatitis for which no causes were apparent, 21 had cryptogenic cirrhosis, and 73 had liver diseases secondary to known causes (eg alcohol, hyperlipidemia, uncontrolled diabetes, Wilson's disease, etc).

Anti-HCV assay

Antibodies against the hepatitis C virus were assayed in the sera of renal failure patients using anti-HCV Elisa test kit from Ortho Diagnostic Systems, USA. The sera of all other patients and that of healthy adults were determined for the presence of anti-HCV using anti-HCV Elisa test kits from Abbotts Laboratory, USA. Positive results were confirmed in the same serum sample and in the case of healthy subjects, in a second serum sample obtained about 4-6 months later. Repeatedly reactive samples were defined as truly positive.

Transaminases were measured at the time of blood taking. Values less than 40 IU/l were regarded as normal.

Statistical analyses

Categorical data were compared using the Chi-squared test with Yates correction.

RESULTS

Healthy subjects

Sera from 463 healthy adults were studied. Their ages ranged from 11-59 years (mean 27 years). Eight (1.7%) subjects were repeatedly positive for anti-HCV. There were 3 men and 5 women with ages ranging from 17-46 years (mean 27 years). All of them had normal transaminase levels except a 39 year old woman who had moderately raised levels of less than 100 IU/l.

Hemodialysis patients

Sera from 112 chronic renal failure patients undergoing maintenance hemodialysis were studied. Their ages ranged from 17-82 years (mean 44 years). Eighteen patients (16%) were HBV carriers. Of the remainder, 50 had evidence of previous HBV infection (anti-HBs and anti-HBc positive), 20 others had hepatitis B vaccination (only anti-HBs positive) and the remaining 24 patients had no markers of HBV infection. Twenty two (20%) of the 112 patients had anti-HCV. There were 12 men and 10 women with ages ranging from 30-55 years (mean 43 years). Only 1 HBsAg carrier (6%) was positive for anti-HCV (Table 1). Anti-HCV was found more frequently among patients with repeated blood transfusions in the older established units than in the newer units (20/67 vs 2/45 $p < 0.005$)

Patients with liver diseases : The sera of 429 patients were studied.

HBV carriers

Five of the 188 carriers (3%) had circulating antibodies to HCV (Table 1). There were 2 women and 3 men with a mean age of 40 years. Three of them had positive serum HBeAg and only a 55 year old man had history of blood transfusion.

Patients with HCC

Anti-HCV was present in 18 of 58 (31%) patients with HCC. There were 14 men and 4 women with a mean age of 60 years. Only one patient had a history of blood transfusion. Of the 58 patients studied, 36 (62%) were HBsAg positive. There was no difference in the seropositivity of anti-HCV among HCC patients who were HBsAg positive compared with those who were HBsAg negative (28% vs 36%) (Table 1).

Patients with other liver diseases (Table 1).

The frequency of anti-HCV positivity in patients with liver diseases not due to hepatitis A, B, cytomegalovirus (CMV), Epstein-Barr virus or

Table 1

Seroprevalence of anti-HCV among normal subjects, patients on hemodialysis and patients with liver diseases in Singapore.

	No. anti-HCV positive (%)	
Healthy subjects (Hepatitis B vaccine trial volunteers)	8/463	(1.7%)
Hemodialysis patients	22/112	(20%)
HBsAg +	1/18	(6%)
HBsAg −	21/94	(22%)
Patients with liver diseases		
Hepatitis B carriers	5/188	(3%)
HBeAg +	3/69	(4%)
HBeAg −	2/97	(2%)
HBeAg not done	0/22	(0%)
Hepatocellular carcinoma	18/58	(31%)
HBsAg +	10/36[a]	(28%)
HBsAg −	8/22[a]	(36%)
Other liver diseases (HBsAg −)	22/183	(12%)
Acute hepatitis	4/34	(12%)
Chronic hepatitis	11/55	(20%)
Cirrhosis	7/21	(33%)
Secondary liver disease	0/73	(0%)
Total	75/1,004	(7.5%)

a; p = NS

drugs was 20% (22/110).

Of the 34 patients who had acute hepatitis not caused by the presently known hepatotropic viruses, 4 (12%) were positive for anti-HCV. Three of them had previous blood transfusion. Anti-HCV appeared 5 months after an acute hepatitis episode in one of them. Two others with previous blood transfusion were negative for anti-HCV. Two patients with associated aplastic anemia were repeatedly negative for anti-HCV during a follow up period of one year. Two other patients with recurrent NANB hepatitis were also repeatedly negative for anti-HCV.

Of 55 patients with chronic liver diseases (persistently elevated transaminases 1.5-2 times normal value for more than 6 months duration), 11 (20%) were positive for anti-HCV. Five of them had histories of previous blood transfusion. Three others with previous blood transfusion history were negative for anti-HCV.

7/21 (33%) patients with cryptogenic cirrhosis had positive anti-HCV. Only one of them had previous blood transfusion. Three others with previous blood transfusion were anti-HCV negative.

Anit-HCV was not detected in the sera of 73 patients with known causes for their liver disease (mean age 46 years). Forty-three patients had fatty liver secondary to hyperlipidemia, 16 patients had alcoholic liver disease, 8 patients had poorly controlled diabetes, 2 patients had drug-induced hepatitis, 2 patients had cytomegalovirus hepatitis, 1 patient had Wilson's disease and 1 had primary biliary cirrhosis.

DISCUSSION

Our results confirm that the hepatitis C virus is

prevalent in this part of the world and appears to be responsible for a proportion of chronic liver diseases not caused by hepatitis B.

The seroprevalence rate among the healthy adult population is slightly high at 1.7%. Data from Europe showed a seroprevalence rate of 0.13-1.2% in healthy blood donors and people in low risk groups (Kuhnl *et al,* 1989, Janot *et al,* 1989, Sirchia *et al,* 1989).

The seroprevalence rate of anti-HCV among hemodialysis patients is higher than that seen in England (Noel *et al,* 1989) and Germany (Roggendorf *et al,* 1989) and similar to the seroprevalence rate in chronic renal failure patients in Spain (Esteban *et al,* 1989). The anti-HCV seroprevalence appears to be higher in the longer established renal dialysis units than in the newer ones. This could be partially explained by equipment sharing and increased frequency of blood transfusion in renal failure patients in these units.

Only 12% of our acute non-A, non-B hepatitis infection group was positive for anti-HCV. This low percentage of anti-HCV positivity could be an underestimate as sera were taken relatively early on in the stage of the disease in most of our patients, when anti-HCV might not have appeared yet (Esteban *et al,* 1989). It is also possible that the absence of anti-HCV does not preclude infection by HCV as the antibody assay system only detect antibodies against a segment of the virus (C-100 3). This was suggested by Weiner and colleagues (1990) who detected HCV RNA by complimentary DNA (cDNA) polymerase chain reaction (PCR) from post-transfusion NANBH clinical samples. They detected HCV RNA in 7/10 patients who had anti-HCV, and in 2/5 patients who did not have anti-HCV. Anti-HCV were determined using kits similar to ours.

Our data also showed that HCV infection is associated with chronic liver disease locally including cirrhosis not caused by the hepatitis B virus. Chronic HCV infection could also be responsible for a proportion of liver cancer in such patients as about 36% of HBsAg negative HCC patients were anti-HCV positive. It is interesting to note that there was no difference in the anti-HCV seroprevalence between HCC patients with HBsAg and those without (28% vs 36%). This could be a reflection of the overwhelming part played by HBV virus in the causation of HCC locally. HCV can act alone or in conjunction with HBV in the pathogenesis of HCC in the local population. Our data are similar to that obtained in South Africa (Kew *et al,* 1990). although the South African data showed that about 57% of patients with HCC not associated with HBsAg were positive for anti-HCV. Data from Europe and Japan suggest that HCV may be a more important cause of HCC than HBV in these areas (Colombo *et al,* 1989, Bruix *et al,* 1989, Sakamoto *et al,* 1988).

There are still many unresolved issues concerning HCV infection. Recent data suggest that patients with anti-HCV are likely to be viremia and that the virus is cytotoxic to liver cells (Weiner *et al,* 1990). Yet 7 of our 8 healthy subjects who were anti-HCV positive had no biochemical evidence of liver damage. It would be of interest to determine whether these patients have compensated cirrhosis or are asymptomatic carriers of HCV. Retrospective testing of serial serum repositories from patients during an outbreak of NANBH in Germany many years ago showed that anti-HCV may occur late after an acute infection. Patients who recovered from their acute illness lost their anti-HCV within 10 years. This together with PCR evidence of viremia in patients positive for anti-HCV suggests that the presence of this antibody does not signify immunity and that it is also not an indicator of previous infection.

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