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• BRIEF REPORTS •

Assessment of correlation between serum titers of hepatitis c virus and severity of liver disease

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Abstract

AIM: The significance of hepatitis C virus (HCV) serum titers has been examined in several clinical situations. There is much evidence that patients with a lower viral load have better response rates to anti-viral therapy compared to those with higher levels. Moreover, a direct association has been observed between serum titers of HCV and transmission rates of the virus. The aim of the present study was to determine if there was any correlation between HCV viral load and the severity of liver disease.

METHODS: Fifty patients with HCV infection were included in the study. These comprised of 34 subjects with a history of alcohol use and 16 non-alcoholics. Quantitative serum HCV RNA assay was carried out using the branched DNA (bDNA) technique. Linear regression analysis was performed between serum viral titers and liver tests. In addition, for the purpose of comparison, the subjects were divided into two groups: those with low viral titers (</=50 genome mEq/mL) and high titers (>50 mEq/mL).

RESULTS: All subjects were men, with a mean±SD age of 47±7.8 years. The mean HCV RNA level in the blood was 76.3×10^5 ±109.1 genome equivalents/mL. There was no correlation between HCV RNA levels and age of the patients (r = 0.181), and the history or amount (g/d) of alcohol consumption (r = 0.07). Furthermore, no correlation was observed between serum HCV RNA levels and the severity of liver disease as judged by the values of serum albumin (r = 0.175), bilirubin (r = 0.217), ALT (r = 0.06) and AST (r = 0.004) levels. Similarly, no significant difference was observed between patients with low viral titers and high titers with respect to any of the parameters.

CONCLUSION: Our results indicate that the severity of liver disease is independent of serum levels of hepatitis C virus. These findings are important since they have a direct impact on the current debate regarding the role of direct cytopathic effect of hepatitis C virus versus immune-mediated injury in the pathogenesis of HCV-related liver damage.

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INTRODUCTION

Hepatitis C virus (HCV) is a bloodborne pathogen that is endemic in most parts of the world, with an estimated overall prevalence of nearly 3%[1]. Approximately 80% patients with hepatitis C virus develop chronic infection, and progression to cirrhosis occurs in nearly 20% of these subjects[2]. Moreover, patients with HCV-related cirrhosis are at an increased risk of developing hepatocellular carcinoma, which is estimated to occur at the rate of 1.5% to 4% per year[2]. In most individuals, liver disease progresses slowly over several decades, but the rate of progression is highly variable[3-5]. Whereas some patients develop cirrhosis and end-stage liver disease within one to two years of exposure, others may die of old age or an entirely unrelated cause[6]. Although it is mostly unclear why some patients progress more rapidly than others, several factors have been identified as having a role in disease severity. HCV patients co-infected with hepatitis B virus (HBV) have an increased risk of developing cirrhosis and ecompensated liver disease[7] as well as hepatocellular carcinoma[8]. Several researches have noted more severe clinical and histological abnormalities in HCV infected chronic alcoholics compared to non-alcoholics with HCV infection[9-12]. Other factors associated with a more rapid course of liver disease include age at acquisition of HCV infection, gender of the patient and presence of immunodeficiency states[5,6].

Several studies have assessed the correlation between serum HCV viral titers and different clinical and laboratory parameters. Perinatal transmission of HCV from mothers to infants has been found to be related to maternal HCV titers. The risk of HCV transmission was found to be significantly higher (36%) among infants born to women with HCV RNA titers of at least 10^6 per mL compared to none if the titers were $<10^6$ per mL[13]. HCV titers have been found to be associated with responses to anti-viral treatment. Patients with a baseline HCV viral load of $</=2\times10^6$ copies per mL have significantly better responses to anti-viral therapy compared to those with higher viral titers[14]. Patients with HCV genotype 1 have been found in some studies to have higher viral loads than those with HCV genotype 2[15,16], although other studies have failed to observe such an association[17-19]. Previous attempts to assess the effect of viral titers on the severity of liver disease have produced conflicting results and the present study was designed to examine this issue in more detail.

MATERIALS AND METHODS

Patients with chronic hepatitis C virus infection diagnosed on the basis of a positive recombinant immunoblot assay (Riba) were included in the study. All patients were negative for other causes of chronic liver disease including hepatitis B virus infection. Patients were interviewed with respect to alcohol use, and in those with a positive history an assessment was made of the duration of alcohol abuse and amount of daily consumption. Physical findings and results of laboratory tests were recorded. All patients were treatment-naive and were tested before the administration of anti-viral therapy.

Quantitative HCV analysis

Quantitative assay of hepatitis C virus levels was performed by the branched-chain DNA (bDNA) technique (Quantiplex

HCV-RNA; Chiron Corporation, Emeryville, USA). The bDNA assay incorporates a series of steps involving viral nucleic acid hybridizations to obtain signal amplification. This technique is unlike the polymerase chain reaction (PCR)-based assays in which the viral genome is amplified. The results of viral RNA titers in clinical samples are expressed as viral or genome milliequivalents per mL (mEq/mL). When the study was first initiated, only the initial version of the bDNA assay (Quantiplex 1.0), which had a lower limit of detection of 350 000 viral mEq/mL, was available commercially. Subsequently, the Chiron Corporation upgraded the technique and the latest version of the assay (Quantiplex 2.0) was employed which has a lower limit of detection of 200 000 viral mEq/mL.

Statistical analysis

Descriptive statistical analyses were performed, and the results are presented as mean±SD. Comparison of quantitative measurements between groups was performed using Wilcoxon Rank Sum Test. The Students' *t*-test was used to assess changes in HCV RNA levels in the same individual. Linear regression analysis was employed to examine the presence of any correlation between serum HCV RNA levels and different laboratory and clinical parameters including the amount of daily alcohol consumption.

RESULTS

A total of 50 patients were included in the study. These comprised of 34 patients with a history of alcohol use and 16 non-alcoholics. All subjects were men, with a mean±SD age of 47±7.8 years. The mean HCV-RNA level in the blood was 76.3×10^5 ±109.1 genome equivalents/mL. There was no correlation between HCV RNA levels and the age of the patients (r = 0.181), a history of alcohol use or the amount (g/day) of alcohol consumption (r = 0.07). Furthermore, no correlation was observed between HCV RNA levels and the severity of liver disease as judged by the values of serum albumin (r = 0.175), bilirubin (r = 0.217), ALT (r = 0.06) and AST (r = 0.004) levels.

To further assess the effects of viral titers on the severity of liver disease, the study subjects were arbitrarily divided into two groups: patients with low viral titers (</=50 genome mEq/mL) and those with high titers (>50 mEq/mL). The results are shown in Table 1. Again, no difference was observed between the two groups with respect to any of the parameters examined.

Table 1 Comparison of patients with low and high hepatitis C virus serum titers

Parameter	Low HCV RNA level (</=50 genome mEq/L) n = 28	High HCV RNA level (>50 genome mEq/L) n = 22	P value
Age (yr)	48±8.8	45±6	0.35
Alcohol use (g/d)	221±110	274±170	0.27
ALT (U/mL)	77±56	75±41	0.76
AST (U/L)	79±58	93±100	0.96
Albumin (g %)	3.70±0.76	3.90±0.43	0.44
Bilirubin (mg %)	1.35±1.2	0.88±0.32	0.55

Results are expressed as mean±SD.

DISCUSSION

Several factors have been incriminated in predicting the rate of progression of HCV-related chronic liver disease. These include age at acquisition of HCV infection, gender of the patient, alcohol abuse and co-infection with HBV and HIV infections[5-12]. Studies assessing the relationship between serum viral titers and the severity of biochemical and histological abnormalities have produced conflicting results. Some found no correlation between HCV viral loads, and serum ALT values and the extent of histological damage[16,17,19-21]. On the other hand, Kato *et al*. observed significantly higher HCV RNA titers in patients with chronic active hepatitis and cirrhosis compared to those with milder histological abnormalities such as chronic persistent hepatitis[22]. Similarly, Fanning *et al*. in a study on Irish women who acquired their HCV infection through the administration of contaminated anti-D immunoglobulin, obtained a significant correlation between serum HCV viral loads and the degree of hepatic inflammation in liver biopsy specimens[23].

In the present study, we further assessed the association between serum HCV RNA titers and several clinical and laboratory factors. Linear regression analysis showed a complete lack of correlation between the viral loads and age at presentation of the patients and the extent of alcohol consumption. Moreover, none of the laboratory tests showed any correlation with HCV viral count. For the purposes of statistical analysis, we subdivided the patients into those with low (</=50 genome mEq/L) and high (>50 genome mEq/L) viral loads. Again, there was no correlation between any of the clinical and laboratory parameters and HCV viral loads (Table 1).

Our results indicate that the severity of liver disease is independent of serum levels of hepatitis C virus. The precise mechanism by which hepatitis C virus damages the liver remains poorly understood. Until recently, a direct cytopathic effect of the virus was considered as the primary form of liver injury caused by the virus. It has been suggested that the degree of liver damage is the result of a complicated interaction between the virus and immune response of the host[24]. Immune mediated liver damage is believed to be initiated by HCV-specific T cells and is enhanced by HCV-induced HLA-A, B and C and intracellular adhesion molecules[25,26]. The results of the present study are important since they argue against a direct cytopathic effect of HCV and support the hypothesis that the pathogenesis of HCV-related liver damage is immune-mediated.

REFERENCES

1 **Wasley A,** Alter MJ. Epidemiology of hepatitis C: Geographical differences and temporal trends. *Sem Liv Dis* 2000; **20**: 1-16
2 **Alter MJ.** The epidemiology of hepatitis C virus in the west. *Semin Liv Dis* 1995; **15**: 5-14
3 **Kiyosawa D,** Soeyama T, Tanaka E. Interrelationship of blood transfusion non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; **12**: 671-675
4 **Tong MJ,** El-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med* 1995; **332**: 1463-1466
5 **Poynard T,** Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C the OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832
6 **Hoofnagle JH**. Hepatitis C: The clinical spectrum of disease. *Hepatology* 1997; **26**(Suppl 1): 15S-20S
7 **Chuang WL,** Chang WY, Lu SN. The role of hepatitis C virus in chronic hepatitis B virus infection. *Gastroenterol Jpn* 1993; 28 (Suppl 5): 23-27
8 **Di Bisceglie AM.** Hepatitis C and hepatocellular carcinoma. *Hepatology* 1997; **26**(Suppl 1): 34S-38S
9 **Coelho-Little ME,** Jeffers LJ, Bernstein DE, Goodman JJ, Reddy KR, de Medina M, Li X. Hepatitis C virus in alcoholic patients with and without clinically apparent liver disease. Alcohol. *Clinical Expt Res* 1995; **19**: 1173-1176
10 **Rosman AS,** Waraich A, Galvin K, Casiano J, Paronetto F, Lieber CS. Alcoholism is associated with hepatitis C but not hepatitis B in an urban population. *Am J Gastroenterol* 1996; **91**: 498-505
11 **Brillanti S,** Masci C, Siringo S, Di Febo G, Miglioli M, Barbara L. Serological and histological aspects of hepatitis C virus in-

fection in alcoholic patients. *J Hepatol* 1991; **13**: 347-350
12 **Zarski JP,** Thelu MA, Moulin C, Rachail M, Seigneurin JM. Interest of the detection of hepatitis C virus RNA in patients with alcoholic liver disease. *J Hepatol* 1993; **17**: 10-14
13 **Ohto H,** Terazewa S, Sasaki N. Transmission of hepatitis C virus from mothers to infants. *N Engl J Med* 1994; **330**: 744-750
14 **McHutchison JG,** Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N Engl J Med* 1998; **339**: 1485-1492
15 **Lau JYN**, Mizokami M, Kolberg JA, Davis GL, Prescott LE, Ohno T, Perrillo RP. Application of six hepatitis C virus genotyping systems to sera from chronic hepatitis C patients in the United States. *J Infect Dis* 1995; **171**: 281-289
16 **Kao JH,** Lai MY, Chen PJ, Hwang LH, Chen W, Chen DS. Clinical significance of serum hepatitis C virus titers in patients with chronic type C hepatitis. *Am J Gastroenterol* 1996; **91**: 506-510
17 **Nousbaum JB,** Pol S, Nalpas B, Landais P, Berthelot P, Brechot C. Collaborative Study Group. Hepatitis C virus type 1b (II) infection in France and Italy. *Ann Intern Med* 1995; **122**: 161-168
18 **Lau JYN,** Davis GL, Prescott LE, Maertens G, Lindsay KL, Qian K, Mizokami M. Distribution of hepatitis C virus genotypes determined by line probe assay in patients with chronic hepatitis C seen at a tertiary referral center in the United States. *Ann Intern Med* 1996; **124**: 868-876
19 **Zeuzem S,** Franke A, Lee JH, Herrmann G, Ruster B, Roth WK. Phylogenetic analysis of hepatitis C virus isolates and their correlation to viremia, liver function tests, and histology. *Hepatology* 1996; **24**: 1003-1009
20 **Lau JY,** Davis GL, Kniffen J, Qian KP, Urdea MS, Chan CS, Mizokami M. Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. *Lancet* 1993; **341**: 1501-1504
21 **McCormick SE,** Goodman ZD, Maydonovitch CL, Sjogren MH. Evaluation of liver histology, ALT elevation, and HCV RNA titer in patients with chronic hepatitis C. *Am J Gastroenterol* 1996; **91**: 1516-1522
22 **Kato NK,** Hosoda K, Ito Y, Ohto M, Omata M. Quantification of hepatitis C virus by competitive reverse transcription - polymerase chain reaction: increase of the virus in advanced liver disease. *Hepatology* 1993; **18**: 16-20
23 **Fanning L,** Kenny E, Sheehan M, Cannon B, Whelton M, O'Connell J, Collins JK, Shanahan F. Viral load and clinicopathological features of chronic hepatitis C (1b) in a homogenous population. *Hepatology* 1999; **29**: 904-907
24 **Rehermann B.** Interaction between the hepatitis C virus and the immune system. *Semin Liv Dis* 2000; **20**: 127-141
25 **Ballardini G,** Groff P, Pontisso P. Hepatitis C virus (HCV) genotype, tissue HCV antigens, hepatocellular expression of HLA-A, B, C, and intracellular adhesion-1 molecules. *J Clin Invest* 1995; **95**: 2967-2975
26 **Nelson DR,** Marousis CG, Davis GL. The role of hepatitis C virus-specific cytotoxic T lymphocytes in chronic hepatitis C. *J Immunol* 1997; **158**: 1473

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