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Correlation Between Serum HCV RNA and Aminotransferase Levels in Patients with Chronic HCV Infection

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Cross-sectional studies on the correlation between serum hepatitis C virus (HCV) RNA and alanine aminotransferase (ALT) levels in patients with chronic hepatitis C have yielded conflicting results. We conducted a longitudinal study to examine the correlation between HCV viremia and serum ALT levels in individual patients over time. Serial samples (mean 9) from 25 patients with chronic HCV infection, including interferon-treated and untreated immunocompetent and immunosuppressed patients, collected over a period of 1–4.8 years (mean 2.6 years) were tested for HCV RNA and ALT levels using a highly reproducible quantitative (bDNA) assay. A significant correlation was found between serum HCV RNA and ALT levels in the patients who received IFN therapy, but no correlation was observed in the untreated patients. Among the untreated patients, the immunosuppressed patients had significantly higher HCV RNA levels (39 ± 4 vs 3.6 ± 8 Meq/ml, $P < 0.0001$) but significantly lower ALT (56 ± 11 vs 97 ± 12 units/liter, $P = 0.03$) levels when compared to the immunocompetent ones. In summary, we found no correlation between serum HCV RNA and ALT levels in chronic hepatitis C patients who are not receiving interferon therapy. Immunosuppression results in higher HCV RNA but lower ALT levels.

KEY WORDS: immunosuppressive therapy; renal transplantation; chronic hepatitis C; interferon.

The pathogenesis of hepatitis C virus (HCV) -induced liver injury remains unresolved. Early studies suggested that HCV may be a cytopathic virus. This is supported by reports that patients with more active or advanced liver disease have higher levels of viremia (1–8). In addition, among patients who receive interferon therapy, a decrease in serum aminotransferase (ALT) levels during treatment is usually accompanied by a parallel fall in serum HCV RNA levels, and a relapse in ALT levels after treatment is accompanied by reappearance of HCV RNA in serum (9–14). However, recent studies found that while immunosuppressive therapy increases serum HCV RNA levels, ALT levels are decreased, suggesting that HCV-induced liver injury is immune-mediated (15–18). Other evidence in support of immune-mediated liver injury in chronic hepatitis C is the frequent presence of lymphoid aggregates in the portal tracts which contain activated B as well as T cells (19, 20) and the recent demonstration of cytotoxic T lymphocytes that react against HCV antigens in liver biopsies (21–27). Furthermore, some studies found no correlation between serum HCV RNA levels and biochemical/histological activity of liver disease (2, 4–7, 28).

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Several factors contribute to the discrepant reports on the correlation between serum HCV RNA and ALT levels. Most reports were based on cross-sectional studies. It is possible that the relationship between serum HCV RNA and ALT levels may differ in different patients depending on the stage of liver disease and immune response of the host. More meaningful data may be generated by studying serial samples from the same patients. While there are many reports on the effects of interferon therapy on changes in serum ALT and HCV RNA levels, very few longitudinal studies have been performed on untreated patients (1, 28). Most of the latter studies employed assays [such as end-point dilution polymerase chain reaction (PCR) assay] that have marked interassay variability and are at best semiquantitative. Owing to the laborious nature of these assays, very few samples at scattered time points were tested for serum HCV RNA levels. Thus, accurate correlation between serum HCV RNA and ALT levels cannot be determined.

The aim of this study was to reexamine the correlation between the level of hepatitis C viremia and activity of liver disease (as determined by ALT level) in patients with chronic HCV infection by testing multiple serum samples using a highly reproducible, quantitative HCV RNA assay (bDNA). To determine whether the relationship between serum HCV RNA and ALT levels is affected by antiviral and immunosuppressive therapy, three groups of patients were studied: untreated and interferon-treated immunocompetent patients and renal transplant recipients who received immunosuppressive therapy.

MATERIALS AND METHODS

Patients. Twenty-five patients (14 men and 11 women), age 23–74 years (mean ± SEM 44 ± 3 years) with chronic HCV infection, who attended the Hepatitis Clinic and the Renal Transplant Clinic in Queen Mary Hospital, Hong Kong, were studied. All patients were anti-HCV positive by second-generation enzyme immunoassays (EIA-2). They included 13 immunocompetent patients and 12 renal transplant recipients. The risk factors for HCV infection were: transfusions in 20 (including eight who had hemodialysis), occupational exposure in one, and unknown in four (Table 1). HCV genotyping data was available in 19 patients showing genotypes 1b in 14, 2b in one, 3a in one, and 6a in three. Eighteen patients had liver biopsies which showed: minimal changes in five, chronic persistent hepatitis in two, chronic active hepatitis in nine, and cirrhosis in two (Table 1). Among the immunocompetent patients, five received interferon-α_{2b} therapy, all had elevated ALT levels and liver biopsies showing chronic hepatitis prior to initiation of treatment. Four patients received 3 MU doses of interferon thrice weekly for six months, the other patient received 3- to 5-MU doses for 12 months. Initial response was defined as normalization in ALT levels at the end of treatment and sustained response as normalization in ALT levels that was maintained for at least six months after treatment. All the renal transplant recipients were anti-HCV-positive prior to transplant, and all received triple immunosuppressive therapy: prednisone, azathioprine, and cyclosporine. All patients were negative for hepatitis B surface antigen (HBsAg) and antibody to human immunodeficiency virus (anti-HIV) and had no other causes of liver disease.

TABLE 1. CHARACTERISTICS OF PATIENTS AT PRESENTATION*

	Immunocompetent		*Immunosuppressed renal transplant*
	Treated	*Untreated*	
Patients (*N*)	5	8	12
Age (yr), mean ± SEM	34 ± 3	55 ± 5	40 ± 3
Sex m/f	2/3	4/4	8/4
Risk factors			
Blood transfusion	4	4	4
Occupational	1	0	0
Transfusion + HD	0	0	8
Genotype			
1b	3	3	8
2b	1	0	0
3a	1	0	0
6	0	1	2
N/A	0	4	2
Liver histology			
Minimal change	0	0	5
CPH	1	0	1
CAH	4	2	3
Cirrhosis	0	2	0
N/A	0	4	3

* HD, Hemodialysis; N/A, not available; CPH, chronic persistent hepatitis; CAH, chronic active hepatitis.

Methods. Between 1990 and 1994, an aliquot of serum was collected from all anti-HCV-positive patients at each clinic visit (every one to six months) and stored at −70°C for HCV RNA and genotype assays. Serial samples, 5–13 (mean 9 ± 0.5), from each patient collected over a period of 1–4.8 years (mean 2.6 ± 0.2 years) were studied. Anti-HCV was tested by EIA-2; HBsAg and anti-HIV were tested by EIA (Abbott Laboratories, North Chicago, Illinois). Serum HCV RNA was detected by a nested reverse transcription-PCR (RT-PCR) assay as described previously (29) and quantitated by the branched-chain DNA assay (Quantiplex bDNA assay, Chiron Corp., Emeryville, California). The detection limit of the assay is 3.5×10^5 viral equivalents per milliliter of serum (0.35 Meq/ml). HCV genotyping was performed using a modification of the Okamoto PCR-based typing assay that can differentiate genotypes 1a, 1b, 2a, 2b, and 3a (30). Additional primers were incorporated to identify genotype 6a, which is the second most prevalent HCV genotype in Hong Kong (31).

Statistical Analyses. An arbitrary value of 0.175 Meq/ml was assigned to serum samples that were undetectable for HCV RNA in the bDNA assay. All analyses on serum HCV RNA levels were performed using logarithmically transformed values. Pearson's test was used to analyze the cor-

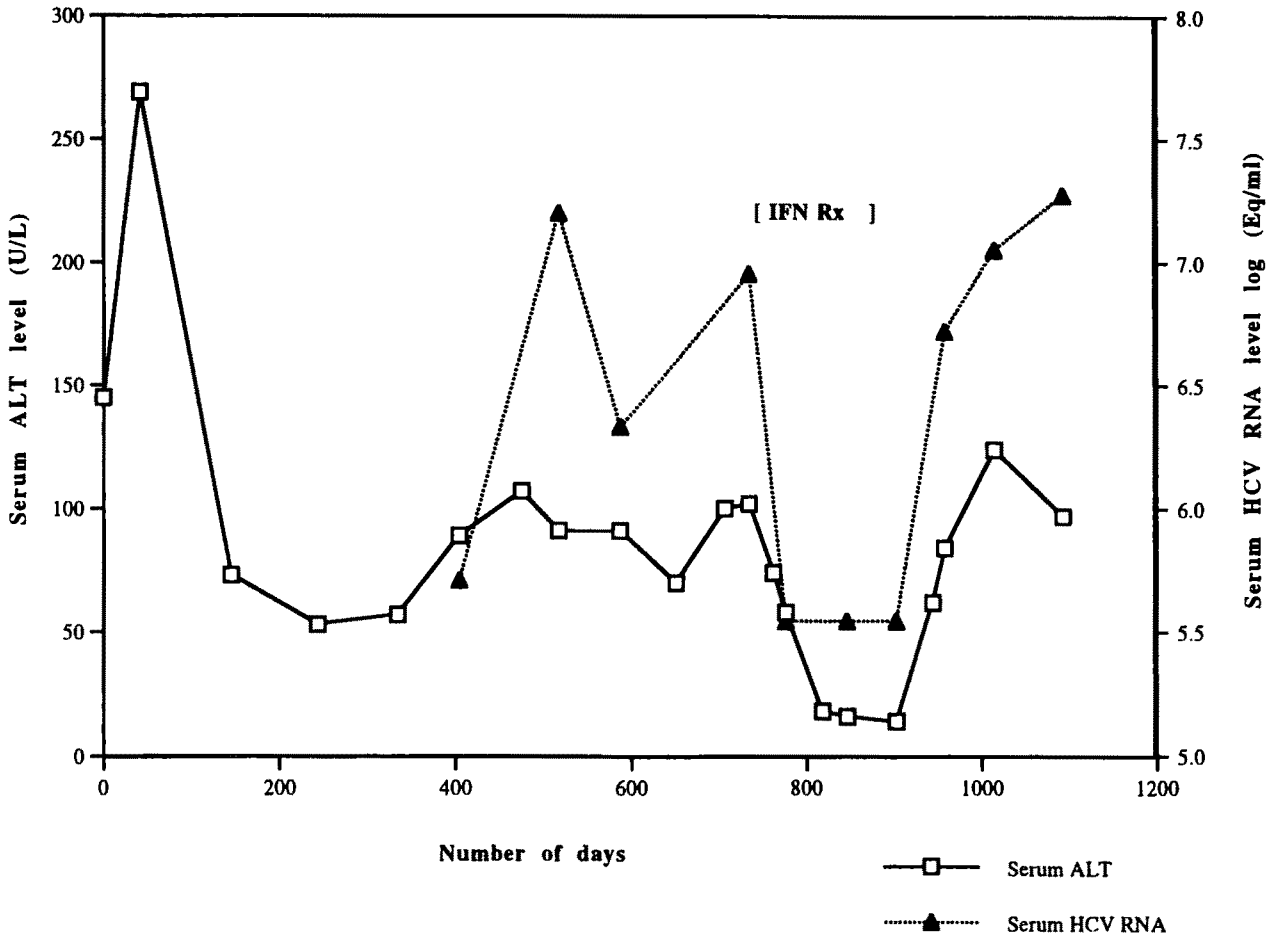

Fig 1. Parallel changes in serum ALT and HCV RNA levels in an interferon-treated patient.

relation between serum HCV RNA and ALT levels. An additional correlation coefficient was calculated for each individual patient by comparing all the paired serum HCV RNA and ALT levels for that patient. In the interferon treated patients, a separate correlation coefficient was calculated for the period from one month before treatment to one month after treatment. Variations in serum HCV RNA and ALT levels during the course of follow-up were calculated by determining the ratio of the peak versus trough HCV RNA and ALT levels for each individual patient. Comparisons between groups were performed using the Mann-Whitney test.

RESULTS

Interferon-Treated Patients (N = 5). All five patients were HCV RNA-positive by RT-PCR, and four (80%) had detectable HCV RNA by the bDNA assay prior to treatment. Initial and sustained response was achieved in four and one patients, respectively. Parallel changes in serum HCV RNA and ALT levels were observed during treatment in the four patients who had detectable HCV RNA by the bDNA assay at the onset of treatment (Figure 1). There was a significant correlation between serum HCV RNA and ALT levels, especially during the treatment period. The mean correlation coefficients and P values for the entire observation period were 0.6 and 0.13 and for the treatment period were 0.91 and 0.02, respectively. All four responders became serum HCV RNA-negative at the end of treatment, but only the sustained responder remained HCV RNA-negative during posttreatment follow-up.

Untreated Patients (N = 20). Of the 20 untreated patients, 12 (three immunocompetent and nine renal transplant patients) were persistently serum HCV RNA-positive by the bDNA assay. Serum HCV RNA was intermittently undetectable in four immunocompetent and three renal transplant patients. Serum HCV RNA was persistently undetectable by the bDNA assay in one immunocompetent patient, al-

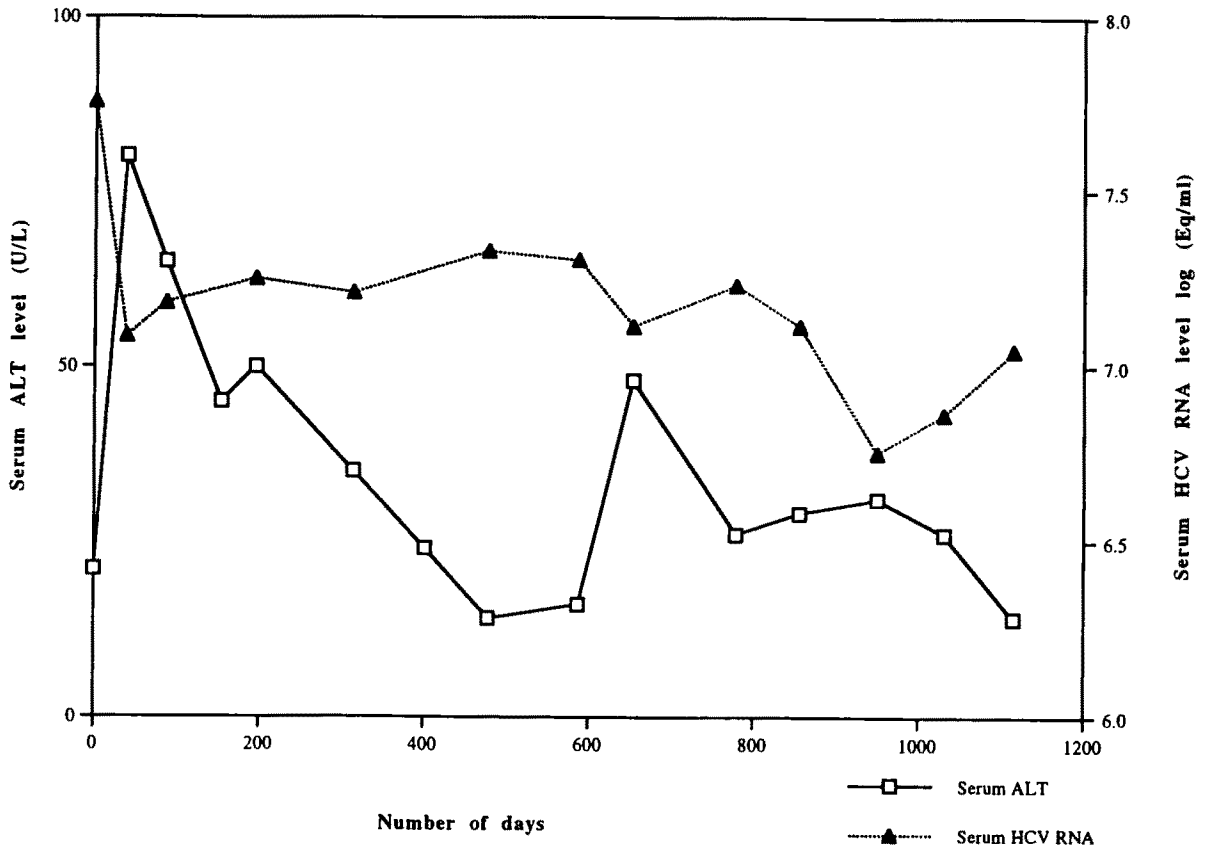

Fig 2. Lack of correlation between serum ALT and HCV RNA levels in an untreated renal transplant recipient.

though he was HCV RNA-positive by RT-PCR. No correlation between serum HCV RNA and ALT levels was observed in any of the 20 untreated patients (Figure 2). The correlation coefficients varied from 0.07 to 0.83 and the *P* values ranged from 0.06 to 0.97.

Compared to the immunocompetent patients, the renal transplant recipients were similar in age: 40 ± 3 vs 55 ± 5 years and had a similar prevalence of HCV genotype 1b: 8 of 10 vs 3 of 4 (Table 1). Despite significantly higher mean serum HCV RNA levels [39 ± 4 vs 3.6 ± 0.8 Meq/ml ($P < 0.0001$)], the renal transplant recipients had significantly lower mean ALT levels: 56 ± 11 vs 97 ± 12 units/liter ($P = 0.03$) (Table 2) and milder histological liver disease: five of nine had minimal changes versus none of four among immunocompetent patients (NS). The renal transplant recipients had fivefold greater variations in serum HCV RNA levels: 43 ± 20 vs 8 ± 2 but a similar degree of variation in ALT levels: 5 ± 1 vs 4 ± 1 (Table 2).

TABLE 2. COMPARISON BETWEEN SERUM HCV RNA AND ALT LEVELS IN UNTREATED IMMUNOCOMPETENT (IC) AND RENAL TRANSPLANT (RT) PATIENTS*

	Untreated patients		
	Immunocompetent	*Renal transplant*	P
Patients (*N*)	8	12	
Points evaluated (*N*)	58	111	
Duration of follow-up (mo)	22 ± 2 (13–32)	39 ± 4 (21–57)	0.03
Mean ALT (units/liter)	97 ± 12 (47–143)	56 ± 11 (17–136)	0.03
Variation in ALT during follow-up	4 ± 1 (1.5–4.8)	5 ± 1 (1.5–14.4)	NS
HCV RNA Level (eq/liter $\times 10^6$)	3.6 ± 0.8 (0.175–39.55)	39 ± 4 (0.175–96)	<0.0001
Variation in HCV RNA during follow-up	8 ± 2 (1–21)	43 ± 20 (2–256)	

* Values expressed as mean ± SEM (range).

DISCUSSION

We previously reported a lack of correlation between serum HCV RNA and ALT levels in chronic hepatitis C patients in a longitudinal study (23). In that study, serum HCV RNA level was quantitated using a semiquantitative assay (end-point dilution RT-PCR assay) that had a 10-fold interassay variability. Considerable controversy exists in the published literature regarding the relationship between levels of hepatitis C viremia and biochemical/histological activity of liver disease in patients with chronic HCV infection (2–6, 23). This is in part related to the poor standardization and interassay variability in amplification efficiency of RT-PCR assays. In this study, multiple samples [5–13 (mean 9 ± 0.5)], at close intervals were tested for serum HCV RNA levels using a more consistent quantitative assay that relies on amplification of enzyme signal rather than nucleic acids. We confirmed our previous finding that except during interferon treatment, there was no correlation between serum HCV RNA and ALT levels in both immunocompetent and immunosuppressed patients with chronic HCV infection. This suggests that HCV-induced liver injury is not a direct result of the cytopathic effects of the virus. Further support against cytopathic liver injury is evidenced by the paradoxical increase in serum HCV RNA and decrease in ALT levels and the milder histological changes in renal transplant recipients receiving immunosuppressive therapy. In addition, while the renal transplant recipients had fivefold greater variations in serum HCV RNA levels compared to the untreated immunocompetent patients, the variations in ALT levels were similar.

In accordance with other investigators, we found significant (up to 14-fold) variations in ALT levels during the course of chronic HCV infection (32, 33). Significant variations including intermittent disappearance in serum HCV RNA levels were also observed in the untreated patients. Intermittent loss of viremia has also been reported by other investigators using more sensitive RT-PCR assays (32, 33). The variability in serum HCV RNA and ALT levels may reflect fluctuations in the balance between host immune response and level of HCV replication and/or emergence of new versus changes in the proportion of existing viral quasispecies.

In summary, we found that there was no correlation between serum HCV RNA and ALT levels during the natural course of chronic HCV infection. Immunosuppression resulted in significantly higher serum HCV RNA but significantly lower ALT levels.

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