J Gastroenterol 1994; 29:593–597

Journal of Gastroenterology

Correlation between serum levels of alanine aminotransferase and ferritin in male blood donors with antibody to hepatitis C virus

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Abstract: Chronic hepatitis C has been demonstrated to be associated with hepatic iron overload, and the hypothesis that the disease activity of hepatitis C is associated with iron cytotoxicity was tested in male volunteer blood donors. Sera with either antibody to hepatitis C virus or hepatitis B surface antigen were selected for determination of ferritin concentration and alanine aminotransferase activity. A correlation between serum ferritin concentration (Y; μg/l) and alanine aminotransferase activity (X; IU/l) was found in donors with antibody to hepatitis C ($\log Y = 0.65 \times \log X + 0.98$, $r = 0.53$, and $P < 0.01$). The correlation was lower in donors with hepatitis B surface antigen ($r = 0.37$; $P < 0.01$). Hepatitis C virus infection probably induces time-dependent iron accumulation associated with the progression of disease activity, while hepatitis B virus infection results in a variety of iron loads with different clinical features. The high disease activity related to hyperferritinemia suggests the presence of iron-induced liver damage in donors with hepatitis C.

Key words: ferritin, iron cytotoxicity, hepatitis B, hepatitis C

Introduction

Chronic hepatitis has been demonstrated to be associated with hepatic iron overload, which is sometimes difficult to differentiate from idiopathic hemochromatosis.[1,2] We have previously reported that iron levels in the livers of patients with chronic hepatitis C (CHC) are higher than those of patients with chronic hepatitis B (CHB).[3] Ferritin and iron absorption seem to be increased in patients with CHC, in comparison to these parameters in patients with CHB and other diseases.[4] Iron cytotoxicity in CHC is the subject of some debate. Farinati et al.[5] suggested that high liver iron levels may trigger steatosis of the liver in patients with CHC. However, Hudes et al.[6] reported no linear correlation between aminotransferases and quantitative iron stores in patients with CHC. To test the hypothesis that the disease activity of CHC is associated with iron cytotoxicity, we analyzed the sera of male blood donors positive for different hepatitis virus markers.

Materials and methods

Between November 1989 and October 1992, about 150000 units of blood was collected from residents of Ishikawa and Aichi Prefectures, in the central area of the main island of Japan. Blood samples were tested for serum markers of hepatitis C virus (HCV) and hepatitis B (HB) virus. Tests included second generation assay systems for antibodies against HCV (anti-HCV), determined by passive hemagglutination (Dainabot, Tokyo, Japan); a test for HB surface antigen (HBsAg), determined by reverse passive hemagglutination (Japan Red Cross, Osaka); and tests for HB e antigen (HBeAg) and HB e antibody (HBeAb), determined by radioimmunoassay (Abbott Laboratories, North Chicago, Ill.). Serum levels of alanine aminotransferase (ALT) were measured with an automated analyzer, and ferritin concentrations were determined by radioimmunoassay. When any of these indices were abnormal, the donated blood samples were usually discarded. The present study was permitted by the ethics committees of our institutes, with the stipulation that the privacy of the donors be preserved. Sera from male donors were studied because men are more susceptible to iron overload than women,

Offprint requests to: T. Takikawa
(Received for publication on Sept. 10, 1993; accepted on Feb. 25, 1994)

who experience periodic blood loss during menstruation. Sera with markers for both HBsAg and anti-HCV were excluded to eliminate the effects of dual infections.

One hundred consecutive sera with anti-HCV of 2^{12} or higher and ALT less than 30 IU/l, and 100 consecutive sera with HBsAg of 2^5 or higher and ALT less than 30 IU/l were selected for comparison between healthy donors with HCV and those with HBsAg. The upper limit of the normal ALT range was set as 30 IU/l, since, at this level, there is significant reduction in the incidence of post-transfusion hepatitis. Differences in age and the traditional indices of iron overload, ferritin concentration and ferritin/ALT, were investigated in the two donor groups.

One hundred eighty-four donors with anti-HCV of 2^{12} or higher and ALT of 30 IU/l or higher, and 68 donors with HBsAg of 2^5 or higher and ALT of 30 IU/l or higher were selected for the comparison of donors with chronic hepatitis caused by different agents. Since our samples were obtained from volunteer blood donors, it can be assumed that most donors with high ALT activity and virus markers would have asymptomatic chronic hepatitis. In the comparative analysis between donors with CHB and those with CHC, parameters included age, ferritin concentration, ALT activity, and ferritin/ALT.

To determine the relationship between serum levels of ALT and ferritin, 50 consecutive healthy donors with HBsAg were combined with 68 donors with CHB as a group of donors with HBsAg, while 152 consecutive healthy donors with anti-HCV were combined with 184 donors with CHC as a group of donors with anti-HCV. Normal distributions of log ferritin and log ALT after the addition of the healthy donors satisfied the requirement for regression analysis.

We also carried out further analysis in two subgroups of donors with CHB: 32 donors with HBeAg-positive CHB, and 32 donors with HBeAb-positive CHB. Four samples were excluded because both HBeAg and HBeAb were negative. For sera with ferritin levels of 1000 μg/l or higher, we calculated the iron saturation index, based on measurements of serum iron levels and total iron-binding capacity performed by standard chemical methods.

Statistics

Values given are means ± SD. Indices obtained from each of the groups were compared using the Mann-Whitney test (two-tailed), unless otherwise stated.

Results

The laboratory data for each group of 100 healthy donors, those with anti-HCV, and those with HBsAg, are summarized in Table 1. Healthy donors with anti-HCV were older than those with HBsAg. Serum ALT activity, ferritin concentration, and ferritin/ALT showed no differences between the two groups.

Laboratory data for the 184 donors with CHC and the 68 donors with CHB are summarized in Table 2. The former were older than the latter. Donors with CHC had higher serum ALT activity than donors with CHB. Neither serum ALT activity nor ferritin concentration was related to the ages of donors with chronic hepatitis. The serum levels of ferritin and the ferritin/ALT ratio were similar in these two groups. The populations of donors with hyperferritinemia and of donors with high ferritin/ALT values are summarized in Table 3. These populations of donors with traditional indices of iron overload and chronic hepatitis were not different in the two groups when analyzed by the χ^2 test.

The correlation between the serum levels of ferritin and ALT differed in donors with anti-HCV and donors with HBsAg (Figs. 1, 2). Pearson's product moment correlation coefficient (r) between log ALT (IU/l) and log ferritin (μg/l) was greater in donors with anti-HCV than in donors with HBsAg.

The data for the two groups of donors, those with HBeAg-positive and those with HBeAb-positive CHB, are summarized in Table 4. Donors with HBeAg-

Table 1. Laboratory data of healthy blood donors with virus markers

Healthy donors[a]	Age (years)	ALT (IU/l)	Ferritin (μg/l)	Ferritin/ALT (μg/IU)
Anti-HCV-positive (n = 100)	38 ± 11	16.2 ± 6.4	75.9 ± 60.0	5.26 ± 4.66
HBsAg-positive (n = 100)	35 ± 11[b]	14.7 ± 5.8	98.5 ± 69.7	7.10 ± 4.48

[a] Defined as those with alanine aminotransferase (*ALT*) activity less than 30 IU/l
[b] Healthy donors with anti-hepatitis C virus (*HCV*) were older than those with hepatitis B surface antigen (*HBsAg*), analyzed by Mann-Whitney test (two-tailed) ($P < 0.05$)

Table 2. Laboratory data of donors with chronic hepatitis

Donor group	Age (years)	ALT (IU/l)	Ferritin (μg/l)	Ferritin/ALT (μg/IU)
Chronic hepatitis C (n = 184)	42 ± 11	68.1 ± 40.0	211.7 ± 266.2	3.17 ± 2.65
Chronic hepatitis B (n = 68)	35 ± 11[a]	64.9 ± 57.1[b]	168.2 ± 128.4	3.22 ± 2.17

[a] Younger than donors with chronic hepatitis C (*CHC*), Mann-Whitney test (two-tailed) ($P < 0.01$)
[b] Lower than donors with CHC, Mann-Whitney test (two-tailed) ($P < 0.05$)

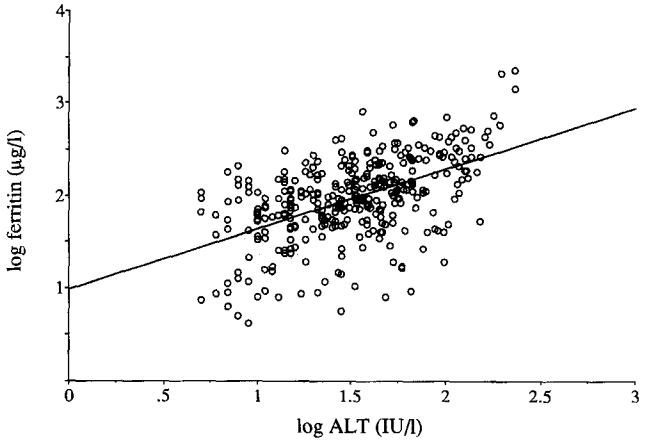

Fig. 1. Good correlation by simple regression analysis between log serum ferritin concentration and log serum alanine aminotransferase (*ALT*) activity in donors with anti-hepatitis C virus. $\text{Log}\, Y = 0.65 \log X + 0.98$; $r = 0.53$; $P < 0.01$

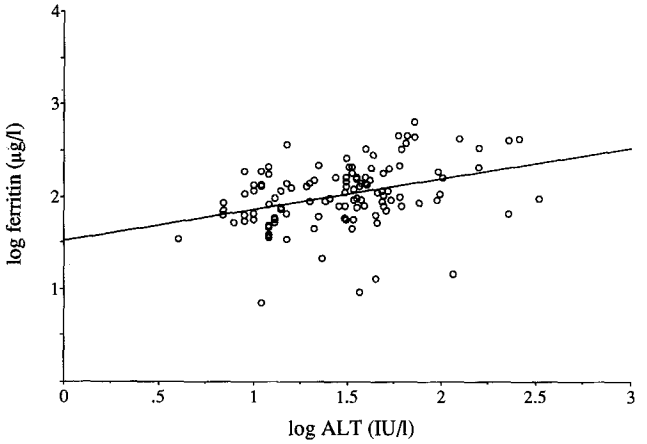

Fig. 2. Poor correlation by simple regression analysis between log serum ferritin concentration and log serum ALT activity in donors with hepatitis B surface antigen. $\text{Log}\, Y = 0.34 \log X + 1.52$; $r = 0.37$; $P < 0.01$

Table 3. Populations of donors with hyperferritinemia and high ferritin/ALT

Donor group	Ferritin (μg/l) <250	Ferritin (μg/l) ≧250	Ferritin/ALT (μg/IU) <5.0	Ferritin/ALT (μg/IU) ≧5.0
Chronic hepatitis C	135	49	155	29
Chronic hepatitis B	55	13[a]	54	14[a]

[a] Not different from donors with CHC, by the χ^2 test

positive CHB were younger than those with HBeAb-positive CHB. Serum levels of ferritin and the ferritin/ALT ratio were higher in donors with HBeAb-positive CHB than in those with HBeAg-positive CHB. The ferritin/ALT ratio was 5.0 or more in six donors with HBeAg-positive CHB and in eight donors with HBeAb-positive CHB. Eight donors with HBeAb-positive mild liver damage, as defined by ALT activity of less than 75 IU/l, had ferritin levels of 250 μg/l or more, while only two donors with HBeAg had such liver damage and hyperferritinemia. The population of these donors, however, was not different when analyzed by the χ^2 test.

Three donors with CHC had serum levels of ferritin higher than 1000 μg/l (Table 5). All three had high levels of ALT and a ferritin/ALT ratio higher than 5.0, with an iron saturation index below 90%.

Discussion

Serum ferritin levels of volunteer blood donors with HBsAg and anti-HCV can be affected by two major factors: the ferritin content of hepatocytes and the severity of hepatocyte necrosis. When necrosis is negligible, serum levels of ferritin are governed by the ferritin content of hepatocytes. Therefore, the serum level of ferritin reflects the iron stored in the body. To reduce the effect of hepatocyte necrosis, the ferritin/ALT ratio can be used instead for the assessment of body iron stores.[7] According to these indices, some donors with HBsAg and anti-HCV in the present study had iron overload. These traditional indices of iron

Table 4. Laboratory data of donors with HBeAg- and HBeAb-positive chronic hepatitis

Subgroup[a]	Age (years)	ALT (IU/l)	Ferritin (μg/l)	Ferritin/ALT (μg/IU)
With HBeAg	31 ± 11	70 ± 66	142 ± 109	2.7 ± 2.0
With HBeAb	38 ± 9[b]	63 ± 51	205 ± 144[c]	3.8 ± 2.3[c]

[a] Each subgroup consisted of 32 donors
[b] Older than donors with HBeAg-positive chronic hepatitis, Mann-Whitney test (two-tailed) ($P < 0.01$)
[c] Higher than in donors with HBeAg-positive chronic hepatitis, Mann-Whitney test (two-tailed) ($P < 0.05$)

Table 5. Iron indices of three donors with anti-HCV and severe hyperferritinemia

Case	Age (years)	ALT (IU/l)	Ferritin (μg/l)	Ferritin/ALT (μg/IU)	Iron (μg/dl)	TIBC (μg/dl)	Saturation (%)
1	36	231	1410	6.1	286	375	76.3
2	38	196	2040	10.4	387	475	81.5
3	45	232	2210	9.5	281	365	77.0

TIBC, total iron-binding capacity

stores, however, do not indicate any differences in iron metabolism between subjects who harbor these two viruses.

Regression analysis of serum levels of ferritin and ALT showed differences in iron metabolism and related liver damage in the two donor groups, those with anti-HCV and those with HBsAg. The good correlation between the two indices (i.e., ferritin and ALT) in donors with anti-HCV, which is a reliable index of the interaction between liver damage and iron overload, indicates three possibilities regarding the underlying mechanisms: (i) virus-induced cell lysis is a major regulator of serum ferritin concentration, (ii) excess iron is a trigger of active cell necrosis, and (iii) the mixed effect of mechanisms (i) and (ii).

Our findings suggest that either natural history or episodes that change the serum ferritin concentration affect ALT activity in donors with anti-HCV. A preliminary study has suggested that HCV stimulates iron absorption in patients with CHC.[4] The 4-year gap between the mean ages of healthy donors with anti-HCV and donors with CHC not only reflects the effects of aging but also reflects time before iron deposits in the liver are manifested clinically. Iron overload during long-term virus infection may exacerbate CHC. Therapeutic effects may be included in the events that reduce serum ferritin concentration. Effective clinical trials in which excess iron was removed from patients with CHC support the possibility of iron-induced liver damage in donors with anti-HCV. Piperno et al.[8] reported that phlebotomy reduced serum levels of aminotransferase in patients with CHC and hyperferritinemia. Repeated phlebotomies can be used to treat CHC; in 4 of 19 patients reported elsewhere,[9] serum levels of aminotransferases returned to normal, without side effects. Thus, it seems most likely that the mixed effect of virus-induced cell lysis and iron cytotoxicity is involved in CHC.

In donors with CHC, however, iron-induced hepatotoxicity does not seem to be important. Infectious liver diseases are associated with iron deposits in the liver through the stimulation of ferritin synthesis.[10] Lustbader et al.[11] have proposed that high serum levels of ferritin result from viral replication in HB. In donors with HBeAg-positive CHB, iron overload differed from case to case, probably because the disease activity is not always time-dependent. Hyperferritinemia was observed in a subgroup of donors with HBeAb-positive mild liver damage, and we speculate that most of these were at a healing stage of chronic active hepatitis in which stage hepatic iron deposits had occurred, and in which liver damage by iron remained, although there was no more viral infection. There was a 7-year gap in the mean ages of donors with HBeAg-positive CHB and those with HBeAb-positive CHB, while there was no such gap between the mean ages of healthy donors with HBsAg and donors with CHB. The traditional indices of iron overload were higher in donors with HBeAb-positive CHB than in those with HBeAg-positive CHB. These observations suggest that HBV stimulates iron absorption and ferritin synthesis. However, it is difficult to say whether iron overload is clinically important in CHB, since the correlation coefficient (determined by regression analysis) between the serum levels of ferritin and ALT in donors with HBsAg was small.

Our study of volunteer blood donors showed a good correlation between serum ferritin concentrations and ALT activity in CHC. Iron metabolism was so complicated in donors with HBsAg that the relationship between serum ferritin concentration and ALT activity was poor in CHB. The high disease activity of CHC related to hyperferritinemia was not due to the presence of aged donors, but to the synergistic effects of iron overload with HCV. The new concept that iron-induced liver damage may occur in donors with anti-HCV is supported by findings that the removal of iron is effective in patients with CHC.

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