Effects of Dietary Iron Reduction Versus Phlebotomy in Patients with Chronic Hepatitis C: Results from a Randomized, Controlled Trial on 40 Japanese Patients

Yoshio Sumida, Kazuyuki Kanemasa, Kohei Fukumoto, Naohisa Yoshida and Kyoko Sakai

Abstract

Background and Aim  Iron may play an important role in the pathogenesis of hepatitis C. We conducted this randomized, controlled trial comparing phlebotomy with dietary iron reduction.

Methods  Forty patients with chronic hepatitis C showing serum ferritin levels of over 150 ng/ml were randomized to either group A (low-iron diet for six months) or group B (phlebotomy biweekly). Phlebotomy was continued until serum ferritin had reached 20 ng/ml or less.

Results  At enrollment the clinical characteristics of patients in the two groups were similar. Serum ALT levels were significantly reduced in both groups, but the percent change in alanine aminotransferase (ALT) was larger in group B (median, -47.1 [range, -69.1 to -16.7] %) than in group A (-24.2 [-72.6 to 15.9] %, p< 0.001). In group A subjects, no correlation was detected between percent change in ALT and clinical parameters. In group B subjects, the baseline ALT activity was significantly correlated with percent change in ALT (p<0.05), but iron-related parameters were not correlated.

Conclusion  The efficacy of phlebotomy is superior to that of dietary iron reduction in chronic hepatitis C. Serum levels of transaminase activities were a better indicator for phlebotomy than conventional indices of iron overload.

Key words: chronic hepatitis C, diet, iron, oxidative stress, phlebotomy

 DOI: 10.2169/internalmedicine.46.6085

Introduction

Hepatitis C virus (HCV) infection has been associated with excess iron deposition in the liver (1, 2). Iron is essential but potently toxic by generating hydroxyl radicals through the Fenton reaction. It has recently been suggested that iron-related oxidative stress may play a role in the pathological mechanisms in chronic hepatitis C (CHC) (2, 3). Several investigators have demonstrated that iron reduction via therapeutic phlebotomy leads to improvements in serum aminotransferase levels in CHC patients (1, 4-6). However, we found few studies which examined the effect of dietary iron reduction on CHC patients. The aims of this randomized, controlled study were to examine whether or not dietary iron restriction improves serum aminotransferase activities and to compare the efficacy of dietary iron reduction with that of phlebotomy in CHC patients.

Materials and Methods

Patients and laboratory evaluation

Forty patients with chronic hepatitis (CH) or liver cirrhosis (LC), type C (31 males and 9 females, age 56 [25-73] years, median [range]), whose serum ferritin levels were over 150 ng/ml, were enrolled in the trial. All patients were positive for serum anti-HCV antibody by a second-generation enzyme-linked immunosorbent assay (ELISA; Ortho Diagnostics, Tokyo, Japan) and for serum HCV-RNA. Serum HCV-RNA was detected by using reverse transcriptional-nested polymerase chain reaction (RT-nested...
PCR) (7). All patients were hepatitis B surface antigen (HBsAg) and human immunodeficiency virus (HIV) negative, and non-drinkers. None of the patients had ingestion of iron supplements, a history of gastrointestinal surgery, hematological disorders, or extensive blood transfusions. Patients with decompensated LC or hepatocellular carcinoma (HCC) were excluded from the study. The diagnosis of CH and LC was performed clinically based on the ultrasonography findings, laboratory data, including indocyanine green clearance test, or histological findings in the liver. We determined routine clinical laboratory parameters, such as alanine aminotransferase (ALT), blood cell count, and ferritin. All the patients showed abnormal serum ALT levels (>30 IU/l) for more than six months before entering the study. The serum level of HCV-RNA was measured by RT-PCR using an AMPLICOR HCV-Monitor Kit (Roche, Tokyo, Japan) (8). The HCV serotype was determined by an ELISA using C14-1 and C14-2 group-specific recombinant peptides from the NS4 region (9).

**Study design**

Subjects were randomized to receive either treatment A, the nutritional education of dietary iron reduction for 6 months, or treatment B, iron removal by phlebotomy biweekly. In subjects randomized to treatment A, daily intake of iron was restricted to less than 7 mg per day under the guidance of a few dietitians at our hospital. Dietitians listened to the details of diet for randomly selected three days and calculated daily intake of iron before the entry according to the fourth revised edition of standard tables of food composition in Japan from the Science and Technology Agency. They presented the iron content in various kinds of food to enhance the knowledge about iron intake, and sometimes listened to the details of diet during the observation period to ascertain whether dietary iron restriction is really maintained. Subjects randomized to treatment B underwent therapeutic phlebotomy of 400 ml of whole blood using standard techniques. Phlebotomy was performed biweekly until serum ferritin levels had reached 20 ng/ml or less. In treatment A group, eight patients had been treated with ursodeoxycholic acid (UDCA), three with UDCA+glycyrrhizin (GL), and the others had been under no medication before entering the trial. In treatment B group, five patients had been treated with UDCA, 5 with GL, 7 with UDCA + GL and others had no medication before entering the trial. These medications were continued throughout the observation period. There were 14 patients (70%) in group A and six (30%) in group B, who had previously been treated with interferon but had not had a sustained response. The mean laboratory values during the 2 months before treatment and the 2 months after treatment were compared. Each mean value was calculated from two to four points obtained during the 2 months. The percentage change in laboratory parameters was determined according to the following equation: Percentage change (%) = [(mean value after treatment-mean value before treatment)/(mean value before treatment)] ×100.

The study design was approved by the Ethics Committee of Nara City Hospital. All patients in this study gave informed consent before entering the study.

**Statistical analysis**

Results are presented as numbers with percentages in parenthesis for qualitative data or as medians and ranges for quantitative data. Statistical differences between 2 groups were determined by the Mann-Whitney U test for quantitative data or χ² analysis for qualitative data (Table 1 and Fig. 2). The statistical analysis in Fig. 1 was performed using the Wilcoxon signed-rank test. Correlation coefficients were calculated using Spearman rank correlation analysis (Table 2). Differences were considered statistically significant at all p values less than 0.05.

**Results**

**Patient demographics**

The clinical, laboratory and demographic features of the patients in the 2 treatment groups at enrollment were comparable (Table 1). Gender, age, clinical parameters, and the proportion of cirrhosis were almost the same in both groups, although the serum HCV-RNA level in treatment A group tended to be higher than in treatment B group without reaching statistical significant differences (p=0.286). In treatment B group, the total volume of blood removed by phlebotomy (median [range]) was 3,200 [1,400-5,200] ml over a period of 4.25 [2.00-10.50] months. The amount of total iron removed by phlebotomy was 1,321 [581-2,426] mg; 1,382 [1,032-2,426] mg in males and 1,082 [581-1,475] mg in females.

**Changes in laboratory parameters**

In treatment A group, sixteen (80%) of 20 patients had decreased serum ALT (Fig. 2A). Only one of them obtained the normalization of serum ALT. Serum ALT levels after dietary iron reduction for 6 months (94 [24-250] IU/l) were significantly lower than before the therapy (126 [31-350] IU/l, p=0.002). In treatment B group, all 20 patients had decreased serum ALT after phlebotomy (Fig. 2B). Two patients obtained normalization of serum ALT. Serum ALT levels after phlebotomy (75 [28-176] IU/l) were significantly lower than before the therapy (142 [40-460] IU/l, p<0.0001). A significant reduction was seen in serum ferritin levels both in group A subjects (448 [240-910] vs. 278 [90-1071] ng/ml, p=0.037) and in group B subjects (445 [152-1795] vs. 15 [5-26] ng/ml, p<0.0001). Group B subjects showed a significant reduction in hemoglobin concentration (15.1 [13.1-18.2] vs. 11.7 [10.3-15.6] g/dl, p<0.0001), while group A subjects showed no reduction in hemoglobin concentration (14.3 [11.9-17.2] vs. 14.3 [12.4-17.8] g/dl, p=0.150). After the therapy, serum ferritin levels and hemoglobin concentrations were significantly lower in group B sub-
Figure 1. Serum alanine aminotransferase (ALT) levels before and after a 6-month course of dietary iron reduction (A) or phlebotomy (B). The significance of difference between before and after each treatment was analyzed by using Wilcoxon signed-rank test: *p=0.002, **p<0.0001, compared to before the treatment.

Table 1. Clinical Characteristics of Study Patients at Enrollment

<table>
<thead>
<tr>
<th></th>
<th>Treatment A (n=20)</th>
<th>Treatment B (n=20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female)</td>
<td>5 (25%)</td>
<td>4 (20%)</td>
<td>.705</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>51 [25-71]</td>
<td>57 [35-73]</td>
<td>.194</td>
</tr>
<tr>
<td>Serum ALT (IU/l)</td>
<td>126 [31-350]</td>
<td>142 [40-460]</td>
<td>.482</td>
</tr>
</tbody>
</table>

HCV serotype

- Group 1: 12 (60%)
- Group 2: 6 (30%)
- unclassified: 1 (5%)
- unknown: 1 (5%)

Serum ferritin (ng/ml)

- Group A: 448 [240-910]
- Group B: 445 [152-1795]

Cirrhosis [n(%)]

- Group A: 1 (5%)
- Group B: 2 (10%)

Results are presented as numbers with percentages in parenthesis for qualitative data, and as medians and [ranges] for quantitative data. P-values for gender and HCV serotype were calculated by χ2 analysis, and the p-values for the other parameters were calculated by Mann-Whitney U analysis with correction for ties. Abbreviations: ALT, alanine aminotransferase.

The comparison of percent change in serum alanine aminotransferase level

The median of percent change in ALT was -24.2 (range -72.6 to 15.9) % in the treatment A group, and -47.1 (range -69.1 to -16.7) % in treatment B group (Fig. 2). A larger decrease in serum ALT was obtained in treatment B group than in treatment A group (p=0.0007).

Correlations between percent change in serum alanine aminotransferase level and clinical parameters

In group A subjects, no correlations between percent change in serum ALT and clinical parameters were found.
Figure 2. Percent change in alanine aminotransferase (ALT) value following phlebotomy to patients with chronic hepatitis or liver cirrhosis, type C. The box comprises the values between the 25th and 75th percentiles and the bold horizontal line is the median; and the error bars stretch from the 10th and to the 90th percentile. The significance of difference between treatment A and B group was determined by using Mann-Whitney U analysis: *p=0.0007, compared to treatment A.

Table 2. Correlation between Percent Change in Alanine Aminotransferase (ALT) and Clinical Parameters in Treatment A and B Groups

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Treatment A</th>
<th>Treatment B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Age</td>
<td>0.209</td>
<td>.362</td>
</tr>
<tr>
<td>ALT (Baseline)</td>
<td>-0.331</td>
<td>.149</td>
</tr>
<tr>
<td>Hemoglobin (Baseline)</td>
<td>-0.172</td>
<td>.453</td>
</tr>
<tr>
<td>Platelet count (Baseline)</td>
<td>-0.061</td>
<td>.791</td>
</tr>
<tr>
<td>Serum HCV-RNA</td>
<td>-0.107</td>
<td>.642</td>
</tr>
<tr>
<td>Serum ferritin (Baseline)</td>
<td>-0.271</td>
<td>.238</td>
</tr>
<tr>
<td>Percent change in serum ferritin</td>
<td>0.311</td>
<td>.175</td>
</tr>
<tr>
<td>Total volume of blood removed by phlebotomy</td>
<td>–</td>
<td>-0.005</td>
</tr>
<tr>
<td>The amount of total iron removed by phlebotomy</td>
<td>–</td>
<td>-0.069</td>
</tr>
</tbody>
</table>

Correlation coefficients (r) and p-values are based on Spearman’s correlation analysis between percent change in ALT and indicated clinical parameters.

Discussion

Several previous studies confirmed that phlebotomy significantly reduces transaminase activities in HCV-infected patients (1, 4–6). This randomized control trial clearly demonstrates that iron removal by phlebotomy is more effective than dietary iron reduction for 6 months, though a longer duration of dietary iron reduction will probably be able to decrease serum ferritin and ALT as well as phlebotomy. The median of percent change in ALT was -47.1% in treatment B group (Fig. 2), so phlebotomy will be one of the most approved therapies for CHC patients who have previously not responded to interferon or other hepatoprotective medica-
In addition to reductions in transaminase activities, platelet count, which is well known to decrease in proportion to the development of hepatic fibrosis, was observed to increase after the completion of phlebotomy. However, we cannot simply draw conclusions that phlebotomy improved hepatic fibrosis, because the platelet count increases with the progression of anemia by stimulating hematopoiesis in the bone marrow. As reported by Yano et al (10), the long-term maintenance of hepatic iron reduction via therapeutic phlebotomy may suppress the development of hepatic fibrosis.

Phlebotomy, often used in western countries to improve hemochromatosis, is not easily acceptable in Japan. One problem is vaso-vagal reaction. Another problem is that a large volume of infectious blood should be disposed. In this way, a few problems remain to be solved for a practical use of phlebotomy. Though iron chelator with deferoxamine may be a useful therapy in thalassemic patients with CHC (11), deferoxamine has been noted to have the deleterious effect on vision or hearing (12). In contrast, phlebotomy showed few harmful side effects, as shown in the present study. Therefore, the administration of deferoxamine is not recommended except for HCV-infected patients with hematological disorders such as thalassemia.

Although the mechanism underlying hepatic iron overload in CHC remains unclear, hepatic excess iron must be originally derived from food. Normally, approximately 10 percent of 10 to 20 mg of iron ingested per day in an average diet is absorbed. This balances the daily obligatory losses of iron from the body that occur mainly from exfoliated gastrointestinal mucosal cells, with smaller amounts lost via the bile, urine, and skin. A recent report demonstrated that dietary iron loading exacerbated liver injury in HCV-infected chimpanzees without influencing viral load (13). Therefore, it is important for the prevention of hepatic iron overload to inhibit iron absorption from gastrointestinal (GI) tracts. The easiest method of reducing iron absorption is the restriction of dietary iron. The most interesting observation obtained in this trial was the fact that even dietary iron restriction significantly decreased serum ALT (Fig. 1). Similar to our findings, a recent study from India (14) has shown that a low-iron diet for 4 months led to significant reductions in both transaminase activities and iron parameters in patients with hepatitis B- or C-related chronic liver diseases. Though the effect of the diet is smaller than that of phlebotomy, this therapy would be more acceptable in countries with cultural backgrounds like Japan. Moreover, low-iron diet, which can be accomplished easily and safely, is recommended as a primary therapy prior to phlebotomy for CHC. Maintenance of hepatic iron reduction via phlebotomy is usually required to keep the state of iron deficiency after the completion of initial phlebotomy, because serum ferritin levels increase gradually after the cessation of phlebotomy by enhancing iron absorption from GI tracts. Therefore, it is probably essential to limit dietary iron intake after completing phlebotomy. Now we are studying the effectiveness of low-iron diet after the cessation of phlebotomy in serum ferritin and transaminase activities.

In Japan, the average intake of dietary iron is 11.7 mg per day according to the national nutrition investigation from the Ministry of Welfare in 1996. We restricted the total quantity of daily dietary iron to less than 7 mg/day irrespective of meal composition, but it will be important to consider what kinds of food should be avoided to obtain dietary iron reduction more efficiently. Iron absorption is regulated via poorly characterized mechanisms to maintain body iron storage at optimum levels. Food iron consists of heme and inorganic iron. Meat should be avoided because meat contains a large amount of heme, which is much more readily absorbed than inorganic iron (15, 16). Inorganic iron constitutes the majority of iron present in the average normal diet, even in Western countries, where meat intake is higher. The bioavailability of inorganic iron is influenced not only by iron status but also by a variety of factors. Absorption of inorganic iron is well known to be aided by gastric acidity (17, 18). Hayashi et al reported that serum ferritin concentrations were stabilized at low levels and sustained normalization of serum transaminase activities were obtained in gastrectomized patients with CHC after stopping phlebotomy (19). These gastrectomized patients have a reduced capacity for iron absorption from GI tracts because of insufficient acidification of ingested nutrients. Therefore, patients with a history of gastrointestinal surgery were excluded from this study. The absorption of inorganic iron is greatly influenced also by dietary compounds. HCV-infected individuals should avoid citrate, ascorbate, and alcohol, which are likely to increase iron absorption (17, 20, 21). Moreover, the supplementation of iron in virtually all commercially prepared foods may have adverse effects on HCV-infected individuals. Conversely, other compounds such as tannins, which form very tight complexes with iron and significantly inhibit iron absorption (22), should be taken positively. As previously shown, tea which contains tannins prevents hemosiderosis in patients with thalassemia (23). Finally, we should pay careful attention to HCV-infected patients with iron-deficient anemia.

As presented in this study, iron removal by phlebotomy or low-iron diet was proved to improve transaminase activities in CHC patients. Until now, an indication of the efficacy of iron removal therapy has never been clarified. The present study implies that serum ALT level may be a good indicator for phlebotomy (Table 2). Unexpectedly, there was no correlation between percent change in ALT, and pretreatment level of serum ferritin, percent change in serum ferritin, total volume of blood removed by phlebotomy, or the amount of total iron removed. This means that hepatic iron stored into ferritin is not necessarily toxic, but that only “free iron” is virulent. Consistent with our result, Hayashi et al revealed that the amount of reduction of serum ALT level is correlated with pretreatment levels of serum ALT, but not iron-related parameters (4). On the other hand, the pretreatment level of serum ALT was not associated with percent change in ALT in group A subjects. These results suggest that phle-
botomy may be recommended for patients with highly active forms of CHC, irrespective of serum ferritin concentrations. In this study, CHC patients with serum ferritin over 150 ng/ml were involved, but patients with normal ferritin concentrations should also be examined in the future.

In conclusion, iron reduction therapy, which is safe and economical, will be prevailing as a form of adjunctive therapy for CHC patients resistant to interferon therapy or hepatoprotective drugs. Though the effect of dietary iron reduction may not be superior to that of phlebotomy, this diet therapy may be a cost effective substitute for phlebotomy and may also have an important role in the management of HCV-infected individuals with iron overload.

References