Efficacy and safety of addition of minor bloodletting (petit phlebotomy) in hepatitis C virus-infected patients receiving regular glycyrrhizin injections

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Running title: Petit phlebotomy for chronic hepatitis C

Abstract

Background: Hepatoprotective therapies that include regular glycyrrhizin injection (GI) are beneficial for chronic hepatitis C patients, but are sometimes unable to normalize serum alanine aminotransferase (ALT) levels. Here, we evaluated whether the addition of minor bloodletting, named petit phlebotomy (PP), prior to each GI could further reduce serum ALT concentrations in such patients.

Methods: Seventy-six HCV-infected patients receiving regular GI with persistently abnormal serum ALT levels were randomly divided into GI+PP or GI groups and monitored for 12 months. PP was performed before every GI to a total 60 ml of blood a week. The primary PP endpoint was serum ferritin levels of less than 20 ng/ml. PP was suspended upon reaching the endpoint, but was resumed as needed. The efficacy of the addition of PP was evaluated by measuring changes in serum ALT levels.

***Results*:** Two patients in each group dropped out because of apparition of hepatocellular carcinoma. The remainder completed the 12-month treatment with no serious adverse events. Serum ALT and ferritin levels were significantly decreased in the GI+PP group (from 67 $\pm$ 34 to 44 $\pm$ 14 U/l and from 163 $\pm$ 127 to 25 $\pm$ 21 ng/ml, respectively, both $P<0.001$), but these changes were not seen in the GI group. Although twenty patients in the GI+PP group had compensated cirrhosis, no significant reductions in serum albumin

concentrations were observed.

Conclusions: The addition of PP is effective and safe for improving serum aminotransferase levels in HCV-infected patients receiving regular GI, even in those with compensated cirrhosis.

Introduction

Chronic hepatitis due to persistent hepatitis C virus (HCV) infection may lead to liver cirrhosis (LC) and eventually hepatocellular carcinoma (HCC).[1] To prevent these complications, maintaining serum alanine aminotransferase (ALT) levels as low as possible is considered very important.[2]

Interferon therapy has been demonstrated to reduce the risk of development of LC and HCC and improve the survival of HCV-infected patients. However, some patients are unwilling to continue interferon therapy because of unpleasant side effects and cost. Furthermore, interferon therapy has the potential to cause serious adverse effects, especially in elderly patients and in patients having arteriosclerosis, thrombocytopenia, or neuropsychological disorders. For such patients, hepatoprotective therapies, including regular intake of ursodeoxycholic acid (UDCA) and/or glycyrrhizin injection (GI), are considered, but are sometimes insufficient to maintain serum ALT levels within normal range.

Recently, the usefulness of iron reduction therapy for HCV-infected patients has been established.[3-7] We hypothesized whether the addition of minor bloodletting, named petit phlebotomy (PP), prior to each GI could adequately reduce serum ALT concentrations, and planned a 12-month randomized prospective study to evaluate this.

Materials and Methods

Patients

From October 2004 to September 2007, 78 HCV-infected patients treated with regular GI in Showa Inan General Hospital and Iida Municipal Hospital were enrolled in this study after obtaining informed consent. All patients were positive for serum HCV-RNA and had demonstrated persistent elevation of serum ALT levels (>45 U/l) for more than 6 months. The exclusion criteria at entry were: (1) previous interferon therapy within 6 months; (2) serum ferritin levels of less than 20 ng/ml; (3) hemoglobin values of less than 11 g/dl; (4) serum albumin concentrations of less than 3.6 g/dl[7]; (5) decompensated LC; (6) malignancy complications or cardiac, pulmonary, renal, or hematological disease; and (7) pregnancy. All patients were treated with UDCA in addition to regular GI for more than 6 months before entry. These agents were not changed after entry. None had received regular administration of branched-chain amino acids, diuretics, or albumin infusion. The enrolled patients were assigned randomly into GI+PP or GI groups using sealed opaque envelopes.

All patients were advised to reduce their intake of iron-rich foods and were counseled by a registered dietitian. To aid with compliance, each patient was given a comprehensive list of iron-rich foods, as well as instructions on how to complete dietary

records, which required a listing of all food and drink consumed over a 3-day period once every 4 months throughout treatment. Iron intake was assessed based on dietary records using the nutrition-analysis software BASIC-4 for Windows version 2.0 (Kagawa Nutrition University Publishing Division, Tokyo, Japan).

Body mass index (BMI) was calculated at entry. Patients were considered to have hypertension if their systolic/diastolic pressure was greater than 140/90 mmHg, or if they were taking anti-hypertensive drugs. Patients were considered to have diabetes if they had a fasting glucose level equal to or higher than 126 mg/dl, or if they were taking insulin or oral hypoglycemic drugs.[8-10]

Diagnosis of LC

The initial diagnosis of LC was made from the histological findings of a percutaneous liver biopsy. In patients who refused liver biopsy, the diagnosis was made using a formula for estimating liver cirrhosis proposed by Ikeda et al.[11] Diagnoses were then further corroborated by imaging findings, such as the presence of liver surface irregularities, swelling of the left or caudal lobes, the presence of splenomegaly, and development of esophageal and/or gastric varices.

Procedure of PP

PP was performed before each regular GI for 12 months using a 24- or 26-gauge needle. The volume of blood drawn was set at a total one-week volume of 60 ml, divided equally by the number of GI given; for example, in patients receiving GI twice a week, 30 ml of blood was removed prior to each GI. The primary PP endpoint was serum ferritin levels of less than 20 ng/ml and the secondary PP endpoint was hemoglobin values of less than 11 g/dl. Upon reaching these endpoints, PP was suspended and conventional hepatoprotective therapies only were continued. If serum ferritin levels began to increase, PP was resumed to again lower them to 20 ng/ml or less. Other discontinuance criteria were the appearance of peripheral edema, ascites, HCC or other serious adverse events, or the patient's refusal to continue PP.

Laboratory examination

Complete blood counts, including hemoglobin values and platelet counts, and biochemical parameters were measured monthly using standard automated analyzers. The amounts of serum HCV-RNA were measured by the Amplicor monitoring method (Roche Diagnostic System, Basel, Switzerland).

Assessment of adverse effects

The presence of any adverse effects from PP was verified prior to every GI by a nursing staff and monitored once a month by a medical interview and physical and blood examinations by a doctor.

Ethics

This study was carried out in accordance with the World Medical Association Helsinki Declaration, and was approved by the ethics committee of the applicable hospitals.

Statistics

Statistical analyses were performed using SPSS software 11.0J for Windows (SPSS Inc., Chicago, Illinois, USA). Qualitative variables were expressed as a percentage and were compared using the χ^2 test. Quantitative data were expressed as mean $\pm$ SD and were compared using the paired or unpaired two-tailed Student's t-test. A *P* value of less than 0.05 was considered to be statistically significant.

Results

Effects of addition of PP

Of the 76 patients enrolled in this study, 2 patients in each group withdrew because of the appearance of HCC. The remaining 72 patients (36 in each group) completed the 12-month treatment. There were no differences in the baseline characteristics between the groups (Table 1). Twenty patients (56%) in the GI+PP group reached serum ferritin levels of less than 20 ng/ml at 7.2 months on average after starting PP. At the end of the study period, significant differences in serum aspartate aminotransferase (AST) and ALT levels, hemoglobin values, and iron profiles, were found between the groups (Table 2). Serum ALT levels were significantly decreased in the GI+PP group after the treatment period (from 67 $\pm$ 34 to 44 $\pm$ 14 U/l, P<0.001) (Figure 1), but remained unchanged in the GI group (from 75 $\pm$ 33 to 71 $\pm$ 28 U/l). Due to the addition of the PP regime, hemoglobin values and serum ferritin levels were also decreased (from 14.0 $\pm$ 1.6 to 12.3 $\pm$ 1.7 g/dl and from 163 $\pm$ 127 to 25 $\pm$ 21 ng/ml, respectively, both P<0.001). Serum albumin, choline esterase (ChE), cholesterol, and α-fetoprotein levels did not differ between the two groups at the end of the treatment course (Table 2).

In chronic hepatitis C patients receiving regular GI, the addition of PP significantly lowered serum ALT and ferritin levels (from 79 $\pm$ 46 to 43 $\pm$ 14 U/l and from 186 $\pm$ 172

to 26 ± 21 ng/ml, respectively, both *P*=0.002) (Table 3).

Effects of addition of PP on compensated LC

The additive effects of PP were also investigated in patients with HCV-related compensated LC. Serum ALT levels, as well as serum AST and ferritin levels and hemoglobin values, were markedly decreased in the GI+PP group after the treatment period (Table 4). These decreases were not seen in 26 patients with LC in the GI group (Table 4). Significant reductions in serum albumin, ChE, or cholesterol concentrations were not observed by the addition of PP (Table 4).

Adverse effects of PP

PP was well tolerated. One patient complained of transient faintness just after PP, but immediately recovered without any treatment. No patients in the GI+PP group showed new signs of peripheral edema or ascites due to decreased serum albumin concentrations throughout the treatment.

Discussion

This study clearly demonstrates the usefulness of addition of PP in HCV-infected patients with persistently abnormal ALT levels, regardless of repeated GI. Long-term GI is believed to be a very effective hepatoprotective therapy, but may sometimes be unable to normalize serum ALT levels. Our results show that the addition of PP to regular GI can further lower serum AST and ALT levels and reinforce hepatoprotection with few adverse effects and at relatively little cost. Thus, PP can be performed in most HCV-infected patients, including elderly patients and those with arteriosclerosis, thrombocytopenia, or neuropsychological disorders.

From the standpoint of iron reduction therapy, this procedure is already considered to be quite useful. In a previous report,[6] regular bi-weekly phlebotomies of 200 or 400 ml were successfully performed in chronic hepatitis C patients until serum ferritin levels reached 10 ng/ml. However, unpleasant symptoms associated with sudden blood loss, such as general fatigue, dizziness, and orthostatic hypotension, may sometimes appear, especially in elderly or female patients. Moreover, the thick needles (16-18 gauge) used in regular phlebotomies often cause stress from puncture pain. On the contrary, a thin needle (24-26 gauge) was used for PP and the volume of blood removed was too small to noticeably affect systemic circulation (20-60 ml). Therefore, we presume that this

method will be easily accepted and adhered to by many patients, even in elderly ones and those with delicate superficial veins. We are now planning a future trial to compare the efficacy and adherence rates between PP and conventional phlebotomy.

It is noteworthy that PP was continued safely for one year in patients with compensated LC. In cirrhotic patients, conventional repeated phlebotomies are sometimes discontinued because of decreases in serum albumin concentrations; some adjustments, such as reduction of removed blood volume or extension of intervals between extractions, are required.[7] On the other hand, the extent of decreases in serum albumin, ChE, and cholesterol levels was minimal in PP. Thus, PP is presumed to be a safe and effective iron reduction therapy for patients with compensated LC as well.

Lastly, it has been reported that long-term intermittent GI successfully reduces the incidence of HCC in patients with HCV-related chronic liver disease.[12] The same results have been found for long-term iron reduction therapy.[13,14] Based on these findings, it is plausible that the combination of GI and PP can reduce the risk of HCC development more than GI alone. A long-term follow-up of the patients enrolled in this study is needed to address this issue.

In conclusion, this study confirmed that addition of PP to regular GI successfully reduced serum aminotransferase and ferritin levels in patients with HCV-related chronic

liver disease. Serious adverse events did not appear from PP, even in patients with compensated LC. For HCV-infected patients with persistently abnormal serum aminotransferase levels in spite of regular GI, the addition of PP just prior to every GI should become a promising therapeutic option.

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References

1. Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, et al. 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.
2. Tarao K, Rino Y, Ohkawa S, Endo O, Miyakawa K, Tamai S, et al. Sustained low alanine aminotransferase levels can predict the survival for 10 years without hepatocellular carcinoma development in patients with hepatitis C virus-associated liver cirrhosis of Child stage A. Intervirology 2004; 47: 65-71.
3. Tanaka N, Kiyosawa K. Phlebotomy: a promising treatment for chronic hepatitis C. J Gastroenterol 2004; 39: 601-603.
4. Kaito M. Molecular mechanism of iron metabolism and overload in chronic hepatitis C. J Gastroenterol 2007; 42: 96-99.
5. Hayashi H, Takikawa T, Nishimura N, Yano M, Isomura T, Sakamoto N. Improvement of serum aminotransferase levels after phlebotomy in patients with chronic active hepatitis C and excess hepatic iron. Am J Gastroenterol 1994; 89: 986-988.
6. Yano M, Hayashi H, Yoshioka K, Kohgo Y, Saito H, Niitsu Y, et al. A significant

reduction in serum alanine aminotransferase levels after 3-month iron reduction therapy for chronic hepatitis C: a multicenter, prospective, randomized, controlled trial in Japan. J Gastroenterol 2004; 39: 570-574.

7. Tanaka N, Horiuchi A, Yamaura T, Komatsu M, Tanaka E, Kiyosawa K. Efficacy and safety of 6-month iron reduction therapy in patients with hepatitis C virus-related cirrhosis: a pilot study. J Gastroenterol 2007; 42: 49-55.
8. Tanaka N, Tanaka E, Sheena Y, Komatsu M, Okiyama W, Misawa N, et al. Useful parameters for distinguishing nonalcoholic steatohepatitis with mild steatosis from cryptogenic chronic hepatitis in the Japanese population. Liver Int 2006; 26: 956-963.
9. Tanaka N, Sano K, Horiuchi A, Tanaka E, Kiyosawa K, Aoyama T. Highly purified eicosapentaenoic acid treatment improves nonalcoholic steatohepatitis. J Clin Gastroenterol 2008; 42: 413-418.
10. Tanaka N, Nagaya T, Komatsu M, Horiuchi A, Tsuruta G, Shirakawa H, et al. Insulin resistance and hepatitis C virus: a case-control study of non-obese, non-alcoholic and non-steatotic hepatitis virus carriers with persistently normal serum aminotransferase. Liver Int 2008; 28: 1104-1111.
11. Ikeda K, Saitoh S, Kobayashi M, Suzuki Y, Tsubota A, Suzuki F, et al. Distinction

between chronic hepatitis and liver cirrhosis in patients with hepatitis C virus infection: practical discriminant function using common laboratory data. Hepatol Res 2000; 18: 252-266.

12. Ikeda K, Arase Y, Kobayashi M, Saitoh S, Someya T, Hosaka T, et al. A long-term glycyrrhizin injection therapy reduces hepatocellular carcinogenesis rate in patients with interferon-resistant active chronic hepatitis C: a cohort study of 1249 patients. Dig Dis Sci 2006; 51: 603-609.
13. Kato J, Kobune M, Nakamura T, Kuroiwa G, Takada K, Takimoto R, et al. Normalization of elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. Cancer Res 2001; 61: 8697-8702.
14. Kato J, Miyanishi K, Kobune M, Nakamura T, Takada K, Takimoto R, et al. Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. J Gastroenterol 2007; 42: 830-836.

Table 1. Baseline characteristics of the patients

	GI+PP (n = 36)	GI (n = 36)	*P*
Age (years)	70 ± 8	68 ± 8	0.310
Male (n)	19 (53%)	13 (36%)	0.236
BMI (kg/m^2)	23.4 ± 3.1	22.0 ± 2.5	0.061
Hypertension (n)	18 (50%)	17 (47%)	1.000
Diabetes (n)	5 (14%)	2 (6%)	0.429
Liver cirrhosis (n)	20 (56%)	26 (72%)	0.220
UDCA intake (mg/day)	558 ± 105	531 ± 128	0.337
Glycyrrhizin injection (ml/week)	111 ± 68	127 ± 54	0.272
Dietary iron intake (mg/day)	7.3 ± 2.1	7.3 ± 1.8	0.840
Hemoglobin (g/dl)	14.0 ± 1.6	13.8 ± 1.6	0.568
Platelet count ($x10^3/\mu l$)	118 ± 44	105 ± 46	0.239
Albumin (g/dl)	4.0 ± 0.3	3.9 ± 0.3	0.676
Bilirubin (mg/dl)	0.7 ± 0.3	0.8 ± 0.4	0.569
AST (U/l)	68 ± 27	70 ± 24	0.707
ALT (U/l)	67 ± 34	75 ± 33	0.201
ChE (U/l)	194 ± 97	170 ± 109	0.520
Cholesterol (mg/dl)	153 ± 26	163 ± 30	0.446
Iron (μg/dl)	131 ± 52	143 ± 56	0.456
Transferrin saturation (%)	39 ± 17	42 ± 21	0.712
Ferritin (ng/ml)	163 ± 127	120 ± 91	0.086
AFP (ng/ml)	26 ± 53	22 ± 27	0.709

Qualitative data are expressed as a percentage and quantitative data are expressed as mean ± SD. *P* values were calculated using either the χ^2 test or the unpaired two-tailed Student's t-test. GI, glycyrrhizin injection; PP, petit phlebotomy; BMI, body mass index; UDCA, ursodeoxycholic acid; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ChE, choline esterase; AFP, alpha-fetoprotein.

Table 2. Characteristics of the patients at the end of the study

	GI+PP (n = 36)	GI (n = 36)	*P*
Total blood phlebotomized (ml)	2206 ± 878		
BMI (kg/m^2)	23.3 ± 3.0	22.2 ± 2.6	0.079
Total glycyrrhizin administered (ml)	5234 ± 3257	5458 ± 2110	0.732
Dietary iron intake (mg/day)	6.0 ± 0.7	6.0 ± 0.6	0.886
Hemoglobin (g/dl)	12.3 ± 1.7	13.7 ± 1.6	**0.003**
Platelet count (x10^3/μl)	133 ± 53	109 ± 46	0.086
Albumin (g/dl)	3.9 ± 0.4	3.9 ± 0.4	0.609
AST (U/l)	52 ± 18	69 ± 24	**0.002**
ALT (U/l)	44 ± 14	71 ± 28	**<0.001**
ChE (U/l)	217 ± 104	229 ± 91	0.781
Cholesterol (mg/dl)	148 ± 28	142 ± 14	0.405
Iron (μg/dl)	81 ± 59	146 ± 68	**0.034**
Transferrin saturation (%)	20 ± 18	43 ± 21	**0.046**
Ferritin (ng/ml)	25 ± 21	131 ± 102	**<0.001**
AFP (ng/ml)	12 ± 12	28 ± 53	0.189

Clinical and biochemical parameters at the end of the 12-month treatment were compared between the GI+PP and GI groups. Quantitative data are expressed as mean ± SD. *P* values were calculated using the unpaired two-tailed Student's t-test. GI, glycyrrhizin injection; PP, petit phlebotomy; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ChE, choline esterase; AFP, alpha-fetoprotein.

Figure Legends

Fig. 1. Changes in serum alanine aminotransferase (ALT) levels.

Data are expressed as mean ± SD. Statistical analysis was conducted using the two-tailed Student's t-test. ■, glycyrrhizin injection (GI) + petit phlebotomy (PP) group (n = 36); ●, GI group (n = 36); *, $P<0.05$ compared with the GI group at the same time point (unpaired t-test); #, $P<0.05$ compared with the baseline in the GI+PP group (paired t-test).