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Outcome of Phlebotomy on Improvement of Liver Enzymes and Steatosis in Non-Alcoholic Fatty Liver Disease: A Randomized, Controlled Trial

Ahmad Hormati[1,2], Ali Khanyabi[2], Sajjad Ahmadpour[2], Faezeh Alemi[2], Seyed Saeid Sarkeshikian[2], Mahboubeh Afifian[3], Abolfazl Mohammadbeigi[4], Mohammad Reza Ghadir[2*]

1. *Dept. of Internal Medicine, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
2. *Gastroenterology and Hepatology Diseases Research Center, Qom University of Medical Sciences, Qom, Iran*
3. *Dept. of Health Information Technology, Tehran University of Medical Sciences, Tehran, Iran*
4. *Dept. of Biostatistics and Epidemiology, School of Health, Environmental Pollutants Research Center, Qom University of Medical Sciences, Qom, Iran*

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Corresponding Information:
Mohammad Reza Ghadir,
Gastroenterology and Hepatology Diseases Research Center, Qom University of Medical Sciences, Qom, Iran
E-Mail: ghadir1968@gmail.com

ABSTRACT

Background & Objective: Iron overload in the liver can potentially induce non-alcoholic fatty liver disease (NAFLD). In this study, we sought to evaluate the phlebotomy in NAFLD, and compare it with modified life style.

Materials & Methods: This randomized, single-blind, clinical trial was carried out to evaluate the efficacy of phlebotomy on liver enzymes and steatosis in NAFLD patients. Forty patients diagnosed with NAFLD were enrolled in the study. Patients were randomized into two groups, including twenty patients in the first group who were under daily consumption of 800 IU vitamin E with modified lifestyle, and the second group who administrated 400 ml phlebotomy at the baseline and fifth month of study alongside the modified lifestyle. Transient elastography (TE) was used to evaluate liver transaminases, hemoglobin, ferritin levels, and liver stiffness prior to and following the intervention. Chi-square and paired t-tests were used to analyze the data, using SPSS v18.

Results: In each group, there were 14 men and 6 women. There was no statistically significant difference in demographic features. After the intervention, the mean liver stiffness of the control group increased from 10.38±2.65 kPa before the treatment to 11.40±6.58 kPa, which was not significantly different (P=0.463). The liver stiffness was 11.29±4.71 kPa in the intervention group before the treatment, which was reduced to 8.10±2.36 kPa after the treatment; however, the difference between pre and post-treatment values was statistically significant (P = 0.009). Before and after the treatment, there were no significant differences in the levels of liver enzymes between the two groups.

Conclusion: Phlebotomy is a useful treatment for NAFLD patients, and decreased liver stiffness as cirrhosis complication.

Keywords: Phlebotomy, Nonalcoholic fatty liver disease, Nonalcoholic steatohepatitis, Transient elastography, Fibroscan

Introduction

In terms of liver disease, non-alcoholic fatty liver disease (NAFLD) is considered as the deposition of triglyceride-based lipid in the liver, over ten percent of liver mass. NAFLD is one of the most frequent liver diseases in developed and developing countries. NAFLD, first identified in 1980, was detected in people who had no history of alcohol consumption, but their pathologic findings were similar to the patients with alcoholic hepatitis. NAFLD involves a wide range of complications from simple steatosis to steatohepatitis and cirrhosis, and in advanced stages might be the etiology of hepatocellular carcinoma (HCC) (1-4). The frequency of NAFLD in the USA and Western Europe is 14%-20%. To the authors' best knowledge, there is no study on the overall incidence of NAFLD in Iran; however, Moghadasifar *et al.,* reported that the prevalence rates of low, moderate, and severe NAFLD in Iran are 33.9%, 26.7%, and 6.6%, respectively (5, 6). It was demonstrated that NAFLD is significantly associated with obesity, hypertension, hyperglycemia, and metabolic syndrome, and these factors increase the risk of the disease (6, 7).

The term *metabolic syndrome* refers to a group of related disorders. Abdominal obesity, poor glucose tolerance, hyperTG, low HDL cholesterol, and/or hypertension are risk factors for cardiovascular disease and are related to metabolic syndrome **(8)**. Heart disease, stroke, and type 2 diabetes mellites are related to metabolic syndrome. It is reported that 25 percent of adults in the U.S are diagnosed with metabolic syndrome **(9, 10)**. Metabolic syndrome has a wide range of clinical manifestations including NAFLD. Nowadays, no FDA medication is approved for NAFLD and effective therapy is urgently needed. However, lifestyle changes, pharmacological agents, surgical approaches, and gut microbiome are involved in the management of NAFLD **(11)**. Since NAFLD has a strong association with metabolic syndrome and obesity, studies show that lifestyle modification and weight loss lead to improved liver histology and decreased liver markers. However, the results of the treatment for liver iron overload, including phlebotomy, are still inconclusive. Some studies show that patients with non-alcoholic steatohepatitis (NASH) have iron overload, but the relationship between NASH and iron overload and the pathogenesis of iron in the development of steatosis is unclear **(12, 13)**. A Phase- 2 clinical trial conducted by Beaton et al., (2013) suggested that phlebotomy improves liver function and histology in patients with NAFLD **(14)**. However, a clinical trial conducted by Adams *et al.,* (2015) suggested that ferritin reduction following phlebotomy does not improve liver enzymes and liver steatosis in patients with NAFLD, but none of the two mentioned studies as well as other studies reported a serious complication for phlebotomy **(15)**.

Various molecular studies suggest iron excess in the development of liver injury; therefore, iron reduction provided by phlebotomy may offer a therapeutic option for NAFLD and metabolic syndrome **(16, 17)**. Phlebotomy is a low-risk and available method for patients without anemia, and since there is no certain consensus on its administration to patients with NAFLD, the current study aimed at evaluating the efficacy of phlebotomy on liver function and stiffness in patients with NAFLD, compared with currently available treatments, including lifestyle modification. The present study used laboratory tests to assess liver function and transient elastography (TE), as a non-invasive and reliable method, to evaluate liver stiffness.

Materials and Methods

The current randomized, single-blinded, clinical trial with a control group was conducted by the Gastroenterology and Hepatology Diseases Research Center, Qom University of Medical Sciences, Qom, Iran.

The target population was all patients with NAFLD referring to Gastroenterology and Hepatology Diseases Research Center in Qom, Iran. The sample size, according to the findings of Luca Valenti's study **(18)** at recovery rate of 67% versus 22%, with a test power of 90% and a type 1 error of 1%, based on the sample size formula was calculated as 20 subjects per group. Fifty patients were screened primarily out of whom, 6 patients were excluded due to unwillingness for phlebotomy, one woman was excluded due to the hemoglobin level below the standard cutoff for the study, and 3 patients were excluded due to cardiovascular and cerebrovascular diseases. All patients with hemochromatosis, viral hepatitis and autoimmune hepatitis were excluded from the study. The remaining 40 subjects with NAFID were allocated to two groups, 20 in each group, by simple random allocation (Figure 1).

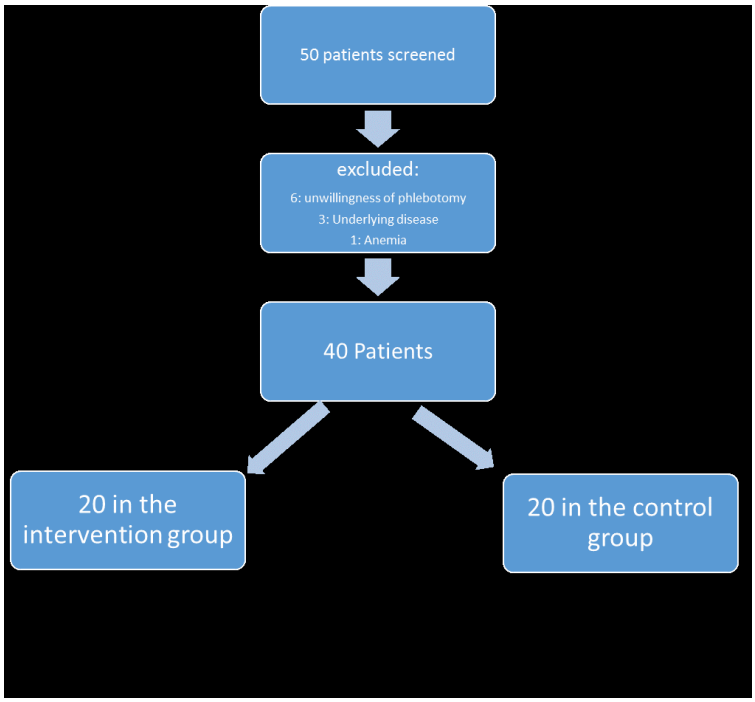

Figure 1. Simple random allocation

Inclusion criteria were the age range of 18 to 65 years, diagnosis of fatty liver disease confirmed by liver ultrasound or transient elastography (TE), hemoglobin level >13 g/dl for male and 12 g/dL for female participants, and willingness to participate in the study and undergoing phlebotomy. Exclusion criteria were decompensated liver cirrhosis or any other liver disease, history of alcohol consumption of more than 20 g daily for male and 10 g daily for female subjects, pregnancy and lactation in female subjects, females of fertility age who did not use a safe contraceptive method, uncontrolled underlying disease, including cardiovascular disease, severe pulmonary disease, seizure, stroke, severe renal disease, and hypertension, weight under 50 kg, having any kind of contraindication for taking vitamin E supplements, and unwillingness to participate in the study.

After explaining the study process and obtaining the signed informed consent form from patients, their demographic data and health information including gender, age, height, weight, blood pressure, history of diseases and drugs they consumed were collected using a checklist. All patients then underwent elastography using the FibroScan® device (FibroScan, Echosens, Paris, France) to assess their liver stiffness at baseline. In this study, one operator who was an expert physician in the field of gastroenterology performed the Fibroscan. When performing fibroscan, the patients had mean fasting time of 4-6 hours Pre-treatment tests including liver aminotransferases, hemoglobin, serum iron, total iron binding capacity (TIBC), fasting blood sugar, and hemoglobin A1c (HbA1C) were performed for all patients, and the results were entered into a checklist for each patient. Then, patients were randomly assigned into two groups. The control group underwent a lifestyle modification program in accordance with the standard guidelines provided by the researchers as well as vitamin E supplementation 800 IU per day. The intervention group, in addition to observing the lifestyle modification guidelines and daily consumption of 800 IU vitamin E, underwent two sessions of phlebotomy in months 0 and 5. Phlebotomy of 400 mL per session was performed using sterile needles and sterile blood transfusion bags. Before and after phlebotomy, the vital signs of patients were monitored. Then, in the 6th month of intervention, laboratory tests plus liver elastography were performed for both groups and the results were recorded in patient's checklist.

Descriptive statistics including frequency, mean and standard deviation were used to express the data. Data analysis was performed using Chi-square test, independent and paired t-test, analysis of covariance, and estimation of correlation coefficient through SPSS version 22.

Ethical considerations: Patients were given information about the study and requested to fill informed consent forms, when they were ready to participate. The Ethics Committee of Qom University of Medical Sciences (ethics code: IR.MUQ.REC.1396.29) approved the study procedures, which were based on Helsinki Declaration. Iranian clinical Trails registry center also registered the study (No. IRCT20161205031252N4).

Results

Of the 40 patients enrolled in the study, 20 (14 male and six female) were allocated to each group. The mean ages of patients in the intervention and control groups were 41.30 ± 10.33 and 40.80 ± 10.16 years, respectively. The means of body mass index (BMI) of the patients in the intervention and control groups were 28.58 ± 1.15 and 29.29 ± 4.57 kg/m^2, respectively. There was no significant difference between the two groups in demographic variables including age (P = 0.875), gender (P = 1.000) and BMI (P = 0.504).

The liver indices measured before and after the intervention in the two groups were liver stiffness (based on FibroScan), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), and alkaline phosphatase. Serum hemoglobin and ferritin were also measured in both groups before and after the intervention. Table 1 shows the mean weight, BMI, liver indices, as well as hemoglobin and ferritin values in both groups before and after the intervention. As indicated in Table 1, there was no significant difference in variables assessed before and after the intervention between the two groups. Figure 2 shows liver stiffness in both groups before the treatment.

Table 1. Patients Demographics and paraclinical findings in both groups, before and after the intervention.

	Before treatment			After treatment		
Variable	**Intervention group**	**Control group**	**P-value**	**Intervention group**	**Control group**	**P-value**
Weight	87.05 ± 9.25	86.35 ± 10	0.819	85.85 ± 9.67	84.95 ± 8.66	0.758
BMI	28.58 ± 1.15	29.29 ± 4.57	0.501	28.17 ± 1.30	28.83 ± 4.20	0.511
Liver stifness	11.29 ± 4.71	10.38 ± 2.65	0.457	8.10 ± 2.36	11.40 ± 6.58	0.045
SGOT	48.90 ± 28.50	56.10 ± 54.31	0.603	43.30 ± 27.16	48.10 ± 32.19	0.613
SGPT	70.05 ± 46.17	68.80 ± 53.06	0.937	62.80 ± 45.17	64.05 ± 49.66	0.934
Alkaline phosphatase	275.65 ± 83.70	242.75 ± 96.97	0.258	270.85 ± 70.45	242.05 ± 95.58	0.285

	Before treatment			After treatment		
Hemoglobine	14.29 ± 1.03	14.24 ± 1.22	0.890	14.09 ± 1.03	14.18 ± 1.19	0.800
Ferritine	147.10 ± 72.34	123.21 ± 62.72	0.272	135.15 ± 68.63	123.75 ± 62.97	0.587

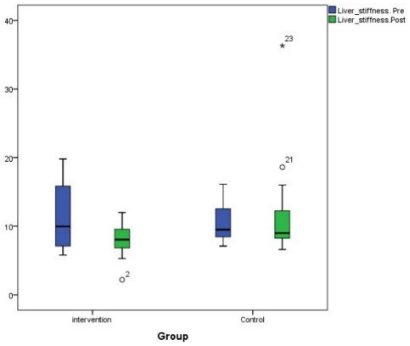

Figure 2. **Liver Stiffness**

The evaluation of liver indices in the intervention group showed that the mean liver stiffness after the intervention decreased from 11.29 ± 4.71 to 8.10 ± 2.36; the difference between pre and post-intervention measures was significant based on the results of paired t-test (P = 0.009). No significant differences were also observed in other liver indices between pre and post-intervention values. The study also showed that the mean serum hemoglobin and ferritin levels decreased significantly after phlebotomy (P <0.001). Table 2 shows the means of the variables assessed in the intervention group before and after the intervention.

Table 2. **Changes in the Intervention group's paraclinical results following the treatment.**

	Before treatment	After treatment	P-value
Liver stifness	11.29 ± 4.71	8.10 ± 2.36	0.009
SGOT	48.9 ± 28.50	43.3 ± 27.16	0.068
SGPT	70.05 ± 46.17	62.80 ± 45.17	0.165
Alkaline phosphatase	275.65 ± 83.70	270.85 ± 70.45	0.330
Hemoglobine	14.29 ± 1.03	14.09 ± 1.03	<0.001
Ferritine	147.10 ± 72.34	135.15 ± 68.63	<0.001

Investigation of hemoglobin and ferritin levels as well as liver indices in the control group before and after the treatment showed that the mean liver aminotransferases decreased following the interventions including lifestyle modification and vitamin E supplementation, although the pre and post-intervention differences were not significant. Also, there was no significant difference in indices such as liver stiffness, hemoglobin, and ferritin. Table 3 summarizes the above- mentioned findings.

Table 3. **Changes in the Control group's paraclinical results following the treatment.**

	Before treatment	After treatment	P-value
Liver stifness	10.38 ± 2.65	11.40 ± 6.58	0.463
SGOT	56.10 ± 54.31	48.10 ± 32.19	0.454
SGPT	68.80 ± 53.06	64.05 ± 49.66	0.580
Alkaline phosphatase	242.75 ± 96.97	242.05 ± 95.58	0.850
Hemoglobin	14.24 ± 1.22	14.18 ± 1.19	0.235
Ferritine	123.21 ± 62.72	123.75 ± 62.97	0.545

The average changes in liver stiffness, as well as the results of liver tests, hemoglobin, and ferritin levels in the two groups are summarized in Table 4. The findings showed that BMI values as well as the levels of liver transaminases, alkaline phosphatase, and hemoglobin in both groups decreased after the intervention, of which hemoglobin decrease was significantly higher in the intervention group compared with that of the control group. The variables of liver stiffness and serum ferritin level decreased in the intervention group and increased in the control group after the treatment. The correlation coefficient between liver stiffness (the differences between pre and post intervention) and ferritin levels (the differences between pre and post intervention) was 0.512 and p value was 0.001 in all patient, while the following

results were obtained for the intervention group: r=0.515 and p value =0.020; and r=0.392 and p value =0.087 for the control group. The difference between the groups was statistically significant.

Table 4. Changes of laboratory values and liver stiffness between the two groups.

Variable	Intervention group	Control group	P-value
BMI	- 0.405 ± 0.660	- 0.465 ± 0.804	0.798
Liver stifness	-3.1950 ± 4.953	1.020 ± 6.087	0.021
SGOT	- 5.60 ± 12.94	- 8.00 ± 46.77	0.826
SGPT	- 7.25 ± 22.44	- 4.75 ± 37.73	0.800
Alkaline phosphatase	- 4.80 ± 21.47	- 0.70 ± 16.33	0.501
Hemoglobine	- 0.20 ± 0.08	- 0.06 ± 0.22	0.015
Ferritine	- 11.95 ± 7.95	0.53 ± 3.88	0.001

Discussion

The prevalence of nonalcoholic fatty liver disease (NAFLD) is rapidly increasing worldwide, and no approved medications exist for managing NAFLD .On the other hand, lifestyle modification can be considered the mainstay for its treatment (19). Liver injury and insulin resistance are commonly presented in patients with NAFLD. The association between hyperferritinemia and the severity of the liver injury has been reported in various studies (20-23). Due to the major role of iron in liver damage and insulin resistance, phlebotomy has been introduced as an iron-depleting therapy in recent years (24, 25). Phlebotomy is a low-risk and available method for patients without anemia. It was demonstrated that its effectiveness in patients with NAFLD is mediated by decreasing insulin resistance and liver transaminase levels, and improving their lipid profile (26).

Few clinical trials have been performed to evaluate the effect of phlebotomy in NAFLD patients, and hence, no certain consensus has been reported on its administration to patients with NAFLD (15, 27-29). In current study, we aimed to investigate the effect of phlebotomy on the improvement of liver markers and liver stiffness in patients with NAFLD. In patients who received a six-month intervention of lifestyle modification only, liver aminotransferases decreased, although it was not significant statistically. Despite recommendations for lifestyle modification in these patients, liver stiffness increased over time which was not significant. Patients who underwent two sessions of phlebotomy, in addition to lifestyle modification, showed a significant improvement in liver stiffness after a six-month intervention. It seems that insulin resistance decreased in these patients due to lower blood sugar levels and improvement in well-being. After the intervention, the mean of liver aminotransferases decreased, although it was not significant statistically. We did not find any significant difference between the two groups in terms of liver enzymes (26). In our study reduction in liver transaminases was not significant. This finding seems unexpected, but is in agreement with the findings of Lavine et al (30).

Oxidative stress may be the underlying pathophysiology of liver damage and fibrosis in patients with iron overload; therefore, phlebotomy can improve the function of the liver in patients with iron overload (12, 31, 32). O'Brien et al., reported that iron accumulation in the liver can elevate synthesis and deposition of fat in the hepatocytes, and phlebotomy can reduce insulin resistance and liver damage in patients with NAFLD (33).

Khodadoostan et al., found that in patients with NAFLD who underwent the 6-month intervention of lifestyle modification, hyperferritinemia was also at high levels (>250 mg/dL). After the 6-month phlebotomy intervention, hemoglobin and ferritin were checked. Findings revealed that hemoglobin level reached 12 in females and 13 in males and significant improvement in liver enzymes and histology were achieved (34). Valenti et al., performed phlebotomy on patients with NAFLD resulting in the reduction of ferritin level (<30 µg/L). Findings revealed that phlebotomy can improve histological liver damage better than lifestyle changes alone in patients with NAFLD (28). For timely treatment of impairments, the hemoglobin levels must be checked routinely in patients who are suspected of NAFLD. Bai et al., findings revealed the comorbidity of NAFLD and iron overload, and hyperferritinemia were found in all patients included in the study; although in our study, patients had a mean ferritin level of <150 ng/ml (35). Despite the possible comorbidity of NAFLD and iron overload, it was reported that patients who are not suffering from iron overload can benefit from phlebotomy. Facchini et al., revealed significant improvement following phlebotomy in patients with clinical evidence of NAFLD without hyperferritinemia, highlighting the beneficial effect of iron depletion in these patients. They reported the insulin-sparing effect of iron depletion and

demonstrated the key role of iron and hyperinsulinemia in the pathogenesis of NAFLD (36). Adams et al., found that phlebotomy had no significant effect on liver enzymes and liver fat content in patients with NAFLD without hereditary mutation in hemochromatosis gene. They believe that the discrepancy between their results and those of other studies can be attributed to different sample sizes, and they suggest that investigation with large sample size may provide the similar results (15). Jaruvongvanich et al., findings revealed the positive effect of phlebotomy on the improvement of liver function and lipid profile, decreasing insulin resistance and liver transaminase levels in patients with NAFLD (37). The reason for discrepancy between the results of their study and those of similar studies as well as our study is unclear, but this difference is interesting and can be considered as a research topic in the future. More extensive studies with larger sample size followed up for longer periods can clarify this contradiction.

Conclusion

Phlebotomy is a low-risk and available method for patients without anemia, and there is no certain consensus on its administration to patients with NAFLD. In current study, phlebotomy as a non-invasive and reliable method was used for improving liver stiffness and elevating liver function in patients with NAFLD, compared with currently available treatments, such as lifestyle modification. Given that phlebotomy removes ferritin, triglycerides and blood sugar in patients, it seems that the procedure may reduce metabolic syndrome and fatty liver without any serious complications. This study gives the ongoing evidence of iron overload on NAFLD pathogenesis that can be used as an introduction for more extensive clinical trials in the future.

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Conflict of Interest

The authors declare that they have no competing interests.

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