Original Article

Effects of Low Dose Zinc Supplementation on Biochemical Markers in Non-alcoholic Cirrhosis: A Randomized Clinical Trial

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Abstract

Background: The present study aimed to evaluate the effects of low dose zinc (Zn) supplementation on biochemical markers and underlying disease status in non-alcoholic cirrhotic patients.

Methods: We enrolled 60 cirrhotic patients in a double-blind, placebo-controlled, randomized clinical trial. All patients in the interventional group (n = 30) received long-term, low dose Zn supplementation (50 mg elemental Zn sulfate daily). The control group (n = 30) received placebo (starch). Child-Pugh scores and biochemical markers were assessed for both interventional and control groups at the first day and the end of the 90th day of the interventional period. A per-protocol analysis was performed after excluding all participants who did not receive or complete the randomized intervention. The mean differences of quantitative variables between and within groups were evaluated by independent samples *t*-test and paired-samples *t*-test, respectively. SPSS version 13.00 was used for statistical analysis.

Results: In the initial evaluation, 16 (53.30%) patients from the interventional group had a Child-Pugh score of 5–8 and 14 (46. 70%) had a score of 9–12. In the control group 18 (60.00%) had a Child-Pugh score of 5–8 and 12 (40.00%) scored 9–12. After three months the mean Child-Pugh score in the interventional group showed a significant improvement (from 6.56 ± 0.21 to 5.72 ± 0.22, $P = 0.001$) whereas in the control group despite no significant decline, the mean Child-Pugh score increased slightly (from 6.25 ± 0.27 to 6.67 ± 0.31, $P = 0.14$). Zn supplementation significantly decreased copper (Cu; $P = 0.01$) and creatinine (Cr; $P < 0.0001$) levels.

Conclusion: In this study, we determined that low dose Zn supplementation could prevent deterioration of clinical status of cirrhosis and prevent excess Cu accumulation in non-alcoholic cirrhotic patients. Zn supplementation produces metabolic effects and trends towards improvements in liver function, hepatic encephalopathy, and nutritional status.

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Keywords: Cirrhosis, clinical trial, zinc supplementation

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Introduction

The liver plays a central role in nutritional homeostasis. Malnutrition is an early, typical aspect of liver cirrhosis which may be an important factor in the development and progression of cirrhosis.[1,2] Malnutrition accounts for more than 60% of patients with severe liver failure and negatively affects clinical outcomes in terms of survival and complications.[3,4]

A consequence of impaired liver function, particularly in patients with cirrhosis, is the change in content of several trace elements in the serum and liver, such as iron (Fe), zinc (Zn), and copper (Cu). The alteration in metabolism of trace elements may be a factor for ongoing liver fibrogenesis and consequently hepatic cirrhosis or hepatocellular carcinoma.[5–7]

The results of some studies suggest that serum Zn levels in patients with liver cirrhosis and hepatocellular carcinoma are significantly lower than in healthy controls.[8–10] Zn deficiency has been implicated in the progression of liver cirrhosis to higher stages.[11] A number of observations have reported significantly higher serum Cu levels in cirrhotic patients than healthy controls.[9,10] According to some authors, a decrease in serum Zn concentration and increase in serum Cu levels could be associated with liver carcinogenesis.[12]

In the present study, the effects of low dose Zn supplementation on biochemical markers and underlying disease status in a number of Iranian non-alcoholic cirrhotic patients have been investigated.

Materials and Methods

Subjects

We assessed 95 patients with histologically documented liver cirrhosis in an outpatient clinic for eligibility. Patients with Child-Pugh scores above 12 (due to unstable condition, overt ascites or necessity for admission), alcoholic cirrhosis, hepatocellular carcinoma, age less than 18 years, use of anabolic hormones, diuretics or albumin (in the past one month), severe renal or respiratory disorders, in addition to patients with clinical evidence of ascites (moderate to severe) or peripheral edema were excluded.

This was a double-blind, placebo-controlled, randomized clinical trial that enrolled 60 patients; 30 were selected randomly as the interventional group which received 50 mg daily oral supplementation of Zn sulfate. The control group (n = 30) received placebo (starch). An informed consent was taken from all 60 patients before enrollment into the study. The randomization was performed using RandList Software. The pharmacist bottled 30 Zn or placebo pills

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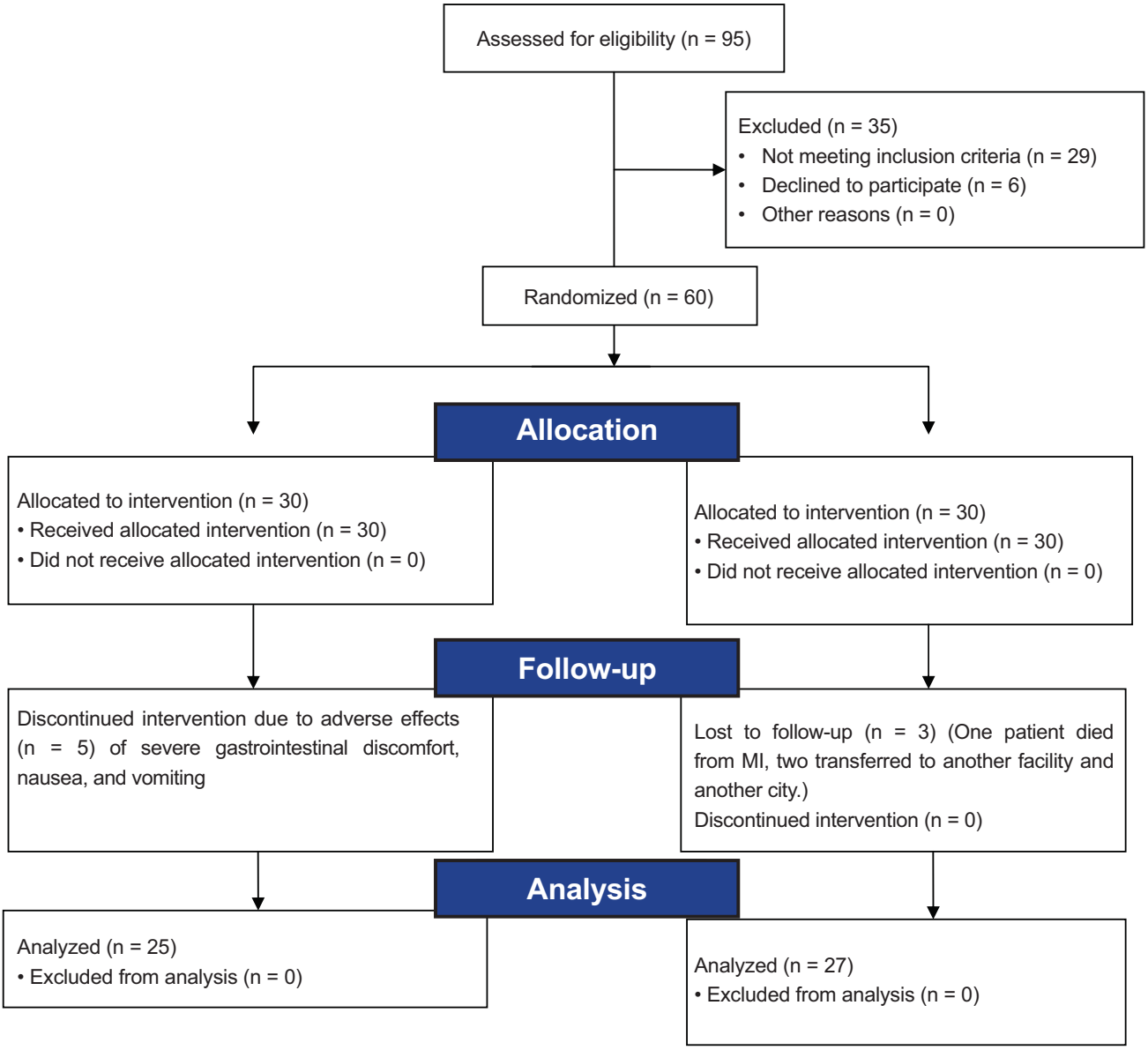

Figure 1. Phases of the Zn supplementation trial.

and coded the bottles with the participants' identification numbers following the randomization code. Only the pharmacist was aware of randomization assignments during the trial. Patients were visited monthly for the acceptability of the supplement, adherence, adverse effects, and morbidity. The bottles of supplements were dispensed monthly to each participant by the pharmacist and the remaining pills counted to assess compliance. Patients who took > 80% of the pills were enrolled in the analysis.

Methods

At the beginning of the study, all patients were assessed by one hepatologist and the severity of liver failure was determined by the Child-Pugh score. Blood samples were collected after an overnight fast (12 hours) at the onset and end of intervention to determine biochemical parameters. The samples were centrifuged and off-the-clot, non-hemolyzed serum samples were removed with a micropipette. The serum samples were kept at -32°C until biochemical analysis.

Serum levels of Zn, Cu, and Fe were determined using flame atomic absorption spectrophotometry (CTA-2000, Chem Tech). Creatinine (Cr) levels were determined by the Jaffe method. Measurements of serum albumin and blood urea nitrogen (BUN) were determined by the calorimetric method quickly after obtaining fasting serum samples. After basal assessments, patients were enrolled into the study and received Zn sulfate capsules (50 mg elemental Zn sulfate daily) or placebo (starch) for 90 days.

Statistical analysis

SPSS version 13.00 was used for statistical analysis. A per-protocol analysis was performed after excluding all participants who did not receive or complete the randomized intervention. The mean differences of quantitative variables between and within groups were evaluated by independent and paired sample *t*-test, respectively. The results were considered significant at the level of $P < 0.05$.

Results

The interventional group consisted of 21 males and 9 females with a mean age of 51.30 ± 15.80 years (range: 19–75 years); the control group consisted of 18 males and 12 females with a mean age of 44.00 ± 15.20 years (range: 19–73 years). Based on the etiology of cirrhosis, 40% of the patients had HBV infection, 13.3% HCV infection, 38.3% autoimmune hepatitis, and 8.3% cryptogenic and other types. During the 90-day period, 5 patients (16.66%) from the interventional group discontinued the study because of severe gastrointestinal discomfort, nausea, or vomiting. In the control group, follow up was discontinued in 3 patients and 27 patients successfully completed the study. Figure 1 shows the flow chart for this study.

The initial evaluation of patients based on the Child-Pugh score revealed that 16 (53.30%) in the interventional group had a Child-Pugh score of 5–8 and 14 (46.70%) had a Child-Pugh score of 9–12. The corresponding values for the control group were 18 (60.00%) that had a Child-Pugh score of 5–8 and 12 (40.00%) whose scores were 9–12. The mean values of the Child-Pugh score and biochemical markers before and after Zn supplementation or placebo administration are shown in Table 1.

Basal levels of the biochemical markers Zn ($P = 0.57$), Cu ($P = 0.80$), serum Fe ($P = 0.58$), albumin ($P = 0.96$), BUN ($P = 0.28$), Cr ($P = 0.84$), and the mean Child-Pugh scores ($P = 1.00$) were similar in paired groups, none of which were significant.

At the end of the trial, the Child-Pugh score improved in the interventional group, whereas in the control group despite no significant decline, the mean Child-Pugh score increased slightly. In two patients who became worse, the Child-Pugh score increased to over 12.

In all subjects, the mean serum Zn concentration was in the lower limit of the normal range. Serum Zn levels increased significantly to 77.87μg/dL (near normal range) after oral Zn supplementation in the interventional group, whereas in the control group no significant change was observed in serum Zn levels at the end of the interventional period (Table 1).

The basal concentration of Cu was found to be in the upper limit of the normal range. Zn supplementation led to a significant decrease in the Cu value, but it remained almost unchanged in the control group. Unlike the control group, the Zn to Cu ratio increased by 31.00% after administration of Zn supplementation in the interventional group.

In general, serum Fe levels in cirrhotic patients were compatible with the reference values.[13] In the control group, serum concentrations of Fe after placebo administration increased insignificantly from 129.14 ± 22.38 to 135.76 ± 28.08 μg/dL in males and from 145.24 ± 21.96 to 174.48 ± 23.14 μg/dL in females. In the interventional group, the mean serum Fe levels reduced slightly in both males (158.29 ± 20.68 vs. 140.18 ± 16.85 μg/dL) and females (151.50 ± 25.62 vs. 150.60 ± 11.64 μg/dL) following Zn supplementation.

There was a significant elevation in the serum levels of albumin after Zn supplementation; however, the albumin levels were not affected significantly by placebo administration (Table 1). BUN concentrations showed a similar result as that observed for albumin (Table 1). Although the serum Cr levels reduced in both Zn supplementation and control groups, this was found to be significant only in the former group.

Discussion

Zn is an essential trace element necessary for a broad range of biological activities such as cell proliferation, normal protein metabolism, membrane integrity, and for the function of more than 200 Zn metalloenzymes.[14–16] Therefore, many of the clinical features of liver cirrhosis have been linked to Zn deficiency including loss of body hair, testicular atrophy, poor appetite, immune deficiency, and distorted protein metabolism.[14–16] We have previously reported that Zn deficiency is observed in cirrhotic patients and its concentration decreases with progression of cirrhosis.[11] The mean protein and energy intake of all patients are lower than RDA values.[11]

Multiple mechanisms have been proposed for Zn deficiency or altered Zn metabolism in patients with liver cirrhosis, including inadequate intake because of early satiety, weakness, fatigue, ascites and low-grade encephalopathy, changes in protein and amino acid metabolism, diminished hepatic extraction, portosystemic shunts, and the influence of cytokines (mainly interleukin-6, IL-6) are known to alter Zn metabolism.[17–19] Zn deficiency may exacerbate the complications of cirrhosis.

In a study by Bianchi et al., a significant improvement in liver function was observed following Zn supplementation in patients with cirrhosis. There was also an improvement in all nutritional indices, although they remained on average below normal.[20] The important role of Zn in the metabolism of trace elements such as Cu and Fe has also been emphasized by some authors.[16,19] These researchers confirmed that oral Zn supplementation produced metabolic effects in Zn-deficient patients with cirrhosis and trends towards improved liver function, hepatic encephalopathy and nutritional status.[16,19]

Trace elements encountered in many protein structures such as metalloenzymes and metallothioneins (MTs).[21,22] The roles of MTs in cells include free metal ion chelation, regulation of the levels and intracellular transport of metals, detoxification of toxic metals such as Cu, and protection of cells against oxidative stress. In addition, these enzymes have an important role in the metabolism of Zn in the liver.[23–25] Various animal studies have shown that Zn supplementation can induce elevated hepatic MT levels.[26,27] It has been shown that oral Zn therapy is useful, inexpensive, and safe in decreasing dietary absorbed Cu as well as Cu that is released from necrotic cells.[28,29] Zn therapy contributes to the detoxification of free Cu through increasing the formation of complexes with Cu-binding proteins such as MT.[22,30]

Fe and Cu act as co-factors of hepatic fibrosis, particularly in collagen synthesis which is the primary cause of chronic liver disease.[30] Numerous research has reported higher Cu and Fe serum levels in patients with liver cirrhosis and hepatocellular carcinoma compared to controls.[6,19,31] The reason for the Fe excess is unknown, but it is possible that acute phase reactions associated with chronic inflammatory states increase uptake of Fe through the gastrointestinal tract leading to removal of excess Fe by Kupffer cells. There is an ineffective erythropoiesis with redistribution of Fe from sites of utilization to sites of storage. Consequently with the damage of hepatocytes, the release of Fe from injured cells to serum develops.[31] It has been shown that Zn treatment can inhibit intestinal Fe absorption and protect the liver from oxidative stress.[32] In this study, we have found that despite low Zn supplementation, serum Cu concentrations significantly reduced but this amount of Zn was not able to significantly decrease Fe levels.

One of the most interesting features concerning the role of Zn

Table 1. Clinical and laboratory data in interventional and control groups of cirrhotic patients before and after intervention.

	NL range[1]	Interventional group (n = 25)			Control group (n = 27)			P-value[4]
		Before zinc (Zn) (mean ± SEM)	After zinc (Zn) (mean ± SEM)	P-value[2]	Before placebo (mean ± SEM)	After placebo (mean ± SEM)	P-value[3]	
Child-Pugh Score	—	6.56 ± 0.21	5.72 ± 0.22	0.001	6.25 ± 0.27	6.67 ± 0.31	0.14	0.01
Zn (μg/dL)	75–291	63.97 ± 4.2	77.87 ± 3.02	0.001	67.42 ± 3.02	64.69 ± 3.16	0.35	0.002
Copper (Cu) (μg/dL)	70–140	136.43 ± 7.03	106.42 ± 4.54	< 0.0001	135.45 ± 7.79	127.35 ± 6.45	0.36	0.01
Zn/Cu ratio	—	0.49 ± 0.04	0.74 ± 0.03	< 0.0001	0.53 ± 0.03	0.52 ± 0.03	0.79	< 0.0001
Iron (Fe) (μg/dL)	50–150	157.11 ± 17.46	142.00 ± 14.00	0.46	136.76 ± 15.39	154.10 ± 18.47	0.34	0.60
Albumin (g/dL)	3.5–5.5	3.88 ± 0.10	4.10 ± 0.08	0.003	4.07 ± 0.11	3.98 ± 0.11	0.01	0.40
Blood urea nitrogen (BUN) (mg/dL)	6–23	14.35 ± 1.44	12.78 ± 0.92	0.22	15.63 ± 1.99	14.47 ± 1.36	0.19	0.20
Creatinine (Cr) (mg/dL)	0.6–1.2	0.92 ± 0.04	0.43 ± 0.04	< 0.0001	0.91 ± 0.05	0.83 ± 0.02	0.63	< 0.0001

[1]: Normal range[13]; [2]: P-value of paired t-test for comparison biochemical markers and Child-Pugh score before and after zinc (Zn) supplementation in interventional group; [3]: P-value paired t-test analysis for comparison biochemical markers and Child-Pugh score before and after placebo in control group; [4]: P-value of t-test for comparison biochemical markers and Child-Pugh score between interventional and control groups at the end of intervention.

in producing clinical features of liver cirrhosis is the possible relationship between Zn deficiency, hepatic encephalopathy, BUN, and serum albumin. The pathogenesis of encephalopathy is still unknown, although a variety of mechanisms such as ammonia and azotemic status have been implicated. Increased ammonia, BUN, and Cr levels in cirrhotic patients with encephalopathy have been observed.[16] In addition, decreases in albumin synthesis aggravate the clinical situation of cirrhotic patients.

The ameliorative effect of Zn supplementation on clinical features of cirrhotic patients is mediated by peripheral mechanisms involved in the reduction of blood ammonia levels.[33] Zn may be involved in the pathogenesis of encephalopathy either by altering nitrogen and ammonia metabolism or by directly influencing brain function.[34,35] Experimental studies on rats have shown that Zn supplementation improves protein synthesis by the liver and influences hepatic fibrosis by increasing hepatic collagenolysis, and by the induction of metallothioneins in the parenchymal areas which protect parenchymal cells against the progression of fibrosis.[35,36] Although the protective effects of Zn supplementation on the structure of the cirrhotic liver in humans has not been studied, it is expected that the improvement of liver structure and function by Zn supplementation can increase albumin synthesis.

Therefore, a reduction in BUN level, improvement of hepatic encephalopathy stage, and increase in albumin levels can lead to a decrease in Child-Pugh score.[37]

Since Cr levels in the serum depend on renal function and muscle mass, in the absence of renal impairment, we have observed that Zn supplementation can significantly decrease Cr levels. This may be due to the improvement of subclinical ascites and muscle mass.

In a double-blind randomized controlled trial, Zn acetate supplementation (200 mg, three times daily, providing a total of 215 mg of elemental Zn per day) was given to cirrhotic patients for seven days, with significant improvements in portal-systemic encephalopathy.[38] Some studies achieved similar results by prescribing 600 mg Zn sulfate for three months.[20,34] This dose of Zn could increase serum Zn concentrations and improve liver function, however, caution should be exercised with regards to the adverse effects of higher doses, because some patients experience abdominal discomfort, nausea and vomiting and deterioration in food and energy intake, and finally clinical status. Therefore, this study has used Zn supplementation at doses of 200 mg daily to reduce the complications seen with higher doses. In comparison to the control group, however, 16.66% of the patients did not tolerate the severe side effects of the 200 mg daily dose of Zn supplementation, yet 83.33% successfully completed the trial with similar results as previous reports.

The papers that we quote for high dose Zn supplementation have not described the number of patients who entered into their studies[20,34,38] Therefore it is not possible to calculate and compare the drop-out rate from these studies.

For the five patients who did not complete the treatment, we were unable to measure the parameters at the end of the study as with the three patients in the placebo group. The missing values of these patients could not be inputed from those who completed the treatment, thus we have used per-protocol analysis which is a weakness in this study. We suggest the prescription of low dose Zn supplementation in cirrhotic patients because of the lack of additional adverse effects versus high dose Zn in improving clinical outcome.

In summary, Zn is an essential micronutrient whose deficiency has been linked to deterioration of clinical features and biochemical indices in cirrhotic patients. Patients with liver cirrhosis frequently have low serum levels of Zn. Because of this, the value of Zn supplementation in patients who are Zn deficient is undisputed. In this study we have found that long-term, low dose Zn supplementation is beneficial on the clinical manifestation and biochemical indices in non-alcoholic cirrhotic patients.

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