Original Article

Effect of Zinc Supplementation on Viral Response in Patients with Chronic Hepatitis C and Beta Thalassemia Major, A Pilot Study

MOHAMMAD ABBASINAZARI[1], SEYED MOAYED ALAVIAN[2], BITA BEHNAVA[3], MANSOUR ASGHARINIA[4], SHIMA SALIMI[5], MARYAM KESHVARI[6], LEALA MEHRNOUSH[7], PEGAH KARIM[8]

ABSTRACT

Introduction: Zinc deficiency has been reported in patients with both hepatitis C and beta thalassemia major. Zinc supplementation in addition to antiviral therapy of chronic hepatitis C has been accompanied by some success in patients with chronic hepatitis C.

Objective: The aim of the present pilot study is to determine the effect of 30 mg elemental zinc on biochemical and virological response in a population of patients with chronic hepatitis C with beta thalassemia major.

Materials and Methods: A prospective, double blind, placebo controlled trial included 40 patients being treated with Pegylated interferon Alfa (Peg IFN-α) and ribavirin. Biochemical and virological parameters and plasma zinc levels were determined before starting treatment. Patients were randomly selected to receive either zinc or a placebo in addition to Peg IFN-α and ribavirin for a period of one year. AST, ALT, sustained viral response (SVR), and zinc levels were measured after treatment. Of the original 40 eligible patients, eight withdrawn from the study and 32 patients completed the study; 16 in the zinc group and 16 in the placebo group. Analysis of the data shows that there is no difference between the two groups in AST, ALT, SVR or zinc level following one year of treatment (p=0.224, p=0.616, p=0.670, p=0.999, respectively).

Conclusion: The results of this study indicate that using 30 mg/day elemental zinc did not significantly improve the outcome of treatment in thalassemia patients with chronic hepatitis C. In future studies, we recommend trying higher doses zinc in patients with hepatitis C who had beta thalassemia major.

INTRODUCTION

Hepatitis C is the most common blood-born pathogen and it is responsible for an estimated 10,000 cases of chronic liver disease annually in the United States [1]. More than 170 million people are infected with hepatitis C virus worldwide [2]. A combination of pegylated interferon Alfa (Peg INF-α) and ribavirin is the treatment of choice for chronic hepatitis C (standard regimen). The target of pharmacotherapy is a sustained viral response (SVR) that shows undetectable serum HCV RNA at 24 weeks after withdrawal of drugs [3]. Current drugs for treatment of chronic hepatitis C mainly affect HCV, including polymerase or protease enzymes [4]. Few investigations have focused on other mechanism of action such as increasing host immunity. Zinc plays a significant role in immune function and zinc deficiency increase susceptibility to various pathogens and prolongs recovery [5]. Zinc homeostasis and signaling are necessary for immune response and an imbalance in zinc homeostasis is accompanied by the development of chronic diseases [6]. However, some authors have reported that zinc administration plays a role in hepatitis C outcome. For the first time zinc deficiency due to interferon has been reported by Gainer [7]. In previous studies, the addition of zinc to the standard therapy for hepatitis C induced limited benefits to viral response and some of these studies have reported improved transaminases with zinc therapy [8]. It is important to note that a group of patients with hepatitis C have beta thalassemia major as well and combination therapy with Peg INF-α and ribavirin should be suggested for them

[9,10]. Hepatitis C infection is a common cause of liver diseases among poly transfused thalassemia [11]. Zinc depletion has been reported in patients with beta thalassemia major under treatment with chelating agents such as deferoxamine, deferiprone, and deferasirox [12]. Use of zinc in addition to the standard regimen may have a benefit in pharmacotherapy of patients with chronic hepatitis C and beta thalassemia major. There is nothing in the literature regarding the effect of the addition of zinc on viral response in patients with hepatitis C with beta thalassemia major. The aim of the present study is to investigate the effects of adding zinc to the standard treatment regimen for these patients.

MATERIALS AND METHODS

For the study, we recruited 40 patients recently diagnosed with chronic hepatitis C and previously diagnosed with beta thalassemia major. Ethical committee approval was obtained from the Baqhatallah University of Medical Sciences before starting the study as per the provision of the Helsinki declaration (2000).The trial was also registered in the Australia and New Zealand Clinical Trial Registry (ANZCTR) with number ACTRN12613000897763.Diagnosis of chronic hepatitis C was based on consistently detectable serum HCV-RNA and serum alanine aminotransferase (ALT), which were at least two-fold above the upper normal limit during the six months prior to starting pharmacotherapy. Diagnosis of beta thalassemia major was based on peripheral blood smear examination and hemoglobin electrophoresis (Hb F >20%) of the patients from
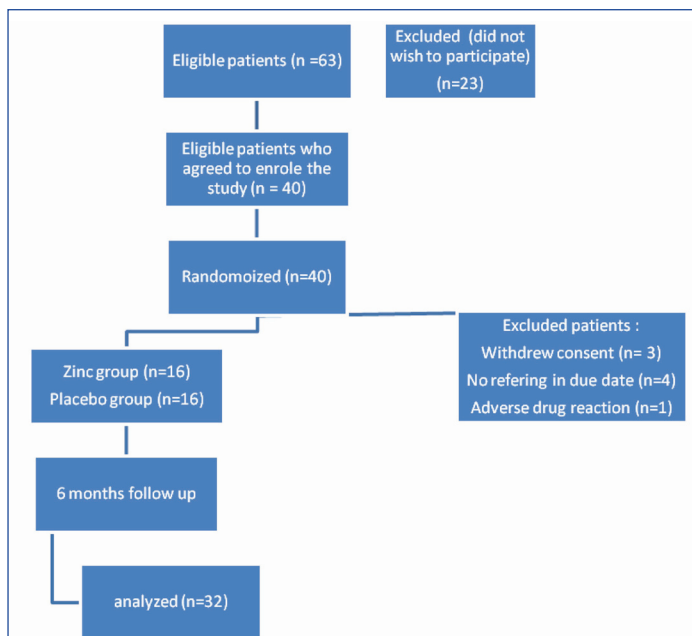
[Table/Fig-1]: Flow of the study

	Zinc group	Placebo group	p-value
Age	26.00±5.83	26.75±4.58	0.689
Sex(Female/Male)	8/8	11/5	0.473
BMI	19.10±2.37	19.81±2.31	0.689
Genotype(1a/3a)	9/7	9/7	0.999
AST(U/L)	85±75	69 ±59	0.402
ALT(U/L)	78 ±52	65±71	0.171
Mean stage of fibrosis	4.35±1.90	4.57±1.34	0.982
Mean grade of inflammation	7.71±3.14	8.50±2.92	0.352
Viral load(log IU/mL)	6.2 ± 2.4	6.8 ± 2.9	0.224
Zinc level (mg/L)	0.75±0.19	0.69±0.17	0.317

[Table/Fig-2]: Baseline demographic, biochemical and biopsy characteristics of studied patients

	Positive response	Placebo group	Total	p-value
Zinc group	13 (81.25%)	3 (18.75%)	16	>0.999
Placebo group	14(87.5%)	2 (12.5%)	16	

[Table/Fig-3]: Rates with virological response in patients with chronic hepatitis C and beta thalassemia major

Parameter	Zinc group	Placebo group	p-value
AST (U/L)	54± 46	48 ± 34	0.616
ALT(U/L)	61± 73	42± 45	0.670
Zinc level (mg/L)	0.84 ± 0.28	0.74 ± 0.15	0.224

[Table/Fig-4]: Liver biochemical parameters and zinc level of 2 groups after ending the pharmacotherapy

ultraviolet spectrophotometric methods on a Hitachi analyzer (Taipei, Taiwan, Roche Diagnostics). HCV RNA was examined by RT-PCR with primers located in the 5'non-coding region of the HCV genome using a commercial kit (Amplicor HCV; Roche Diagnostic, Basel, Switzerland). If HCV RNA could not be detected in serum after the 24 wk follow-up period, the patient was classified as a complete responder to treatment. Those patients who had detectable HCV RNA 24 wk after treatment were classified as non-responders. Plasma zinc levels were determined using the atomic absorption method (Perkin Elmer 1100B). The results are shown as mean ± SD. Data were analysed using SPSS-12 and p-values less than 0.05 was considered statistically significant.

RESULTS

This study was conducted between October 2011 to December 2012 in the Tehran Hepatitis Clinic (affiliated to Baqhatallah University of Medical Sciences), a well-known center for diagnosis and treatment of hepatitis in Iran. Of 40 patients who gave consent for participation in the study, eight did not complete the study due to various causes, most commonly withdrawal of consent,or adverse drug reactions related to the zinc(acute dyspepsia).The flow of the participants through the study is shown in [Table/Fig-1].

A total of 32 patients completed the entire one year study, 16 in the zinc group and 16 in the placebo group. Demographic data such as age, sex, body mass index (BMI), genotype variation, HCV viral load, AST, ALT, stage of liver damage, and zinc level were compared in the two groups. [Table/Fig-2] shows baseline demographic, biochemical, and biopsy data for the two groups.

The two groups were comparable at baseline with respect to demographic characteristics. Sustained viral response (SVR) was defined as undetectable HCV RNA at 24 wk after withdrawal of pharmacotherapy. In the zinc treated group, 81.2% showed SVR, whereas SVR was shown in 87.5% of the placebo treated group. [Table/Fig-3] shows the distribution of responders and non-responders in each group.

Using the chi-square test, there is no significant difference in SVR between the two groups (p>0.999). Serum values of liver biochemical parameters (AST and ALT) were determined at the end of pharmacotherapy and mean values were calculated. The values of liver biochemical parameters are shown in [Table/Fig-4] for the two groups separately.

Using the t-test, there is no significant difference in either AST or ALT between the two groups (p =0.616 and 0.67, respectively). Zinc levels at the end of pharmacotherapy were 0.84 ± 0.28 mg/L in the zinc group and 0.74 ± 0.15 mg/L in the placebo group, which is not a significant difference (p = 0.224). Comparison of zinc levels in both groups before and after the study did not show any significant difference (p = 0.274 in the zinc group and p= 0.218 in the placebo group) either.

the early years of life. All the selected patients were being treated with Peg INF-α 3×10^6 units three times per week, and ribavirin 1000 mg for patients less than 75 kg body weight or 1200 mg for patients over 75 kg body weight for hepatitis C and were being treated with deferoxamine for beta thalassemia major. All patients were negative for hepatitis B and human immunodeficiency virus. Baseline demographic, clinical and paraclinical data were obtained at the start of the study. For determination of baseline biochemical parameters including Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), HCV RNA level, and plasma zinc level, a blood samples were obtained. The mean stage of fibrosis and mean grade of inflammation were also recorded at the start of the study. Informed consent was obtained from patients who agreed to enroll in the study after a thorough explanation of the aims, risks, and benefits of this study. Studied patients were asked to report any adverse drug reactions during the study for decision of continuing the study.

Patients randomly assigned to two groups; the zinc group (Peg INF-α, ribavirin, and zinc) and a control group (Peg INF-α, ribavirin, and placebo). Randomization was conducted by blocks of four and performed by an educated student not involved in the evaluation or treatment of selected patients. Zinc gluconate tablets from Nature Med (equivalent to 30 mg of zinc) were administered to those in the zinc group for 24 wk. Patients were evaluated every two months during the trial. 24 wk after pharmacotherapy ending for hepatitis C (48 wk in genotype 1a and 24 wk in genotype 3a), another venous blood sample was collected. ALT and AST were measured by

DISCUSSION

Serum zinc level has been found to be low in the patients with hepatitis C [13-15]. On the other hand, several mechanisms have been suggested indicating that zinc may improve the response to standard anti-HCV pharmacotherapy. Antioxidant function, promotion of the antiviral effect of interferon, an inhibitory effect on HCV replication, and regulation of imbalances between T helper 1 (TH1) and T helper 2 (TH2) have been considered as probable mechanisms for the effectiveness of zinc in treatment of HCV infection [16,17]. In recent clinical studies, the addition of zinc to the standard treatment regimen for chronic hepatitis C produced limited benefits [8]. For example, Murakami et al., evaluated the addition of polaprezinc 150 mg/d to the combination of Peg INF-α and ribavirin in 21 patients with hepatitis C in a randomized study. Results showed that, 24 wk after withdrawal of pharmacotherapy, all patients in the zinc group and 67% of the control patients showed a decrease in serum ALT levels to within the normal range. Statistical analysis showed a significant difference between two groups regarding ALT decrease after 24 weeks (p< 0.05) [18]. Ko et al., have evaluated the effect of zinc administration in 40 subjects with a diagnosis of hepatitis C. Patients were randomly assigned to receive Peg INF-α /ribavirin with or without zinc gluconate (a dose of 50 mg elemental zinc) for 24 wk. They reported no difference between two groups regarding SVR [19]. Suzuki et al., evaluated the effect of Polaprezinc in 102 patients with confirmed chronic hepatitis C. In their study, patients were randomly assigned to each group and received 10 million units of Peg INF-α daily for 4 weeks followed by the same dose every other day for 20 wk. Patients also received ribavirin, with or without polaprezinc (150 mg/d), orally for 24 wk. Results showed that addition of zinc to Peg INF-α and ribavirin treatment for HCV patients with genotype 1b and high viral load does not improve outcomes, except for a lower incidence of gastrointestinal adverse effects [20].

In the present study we have evaluated the effect of the administration of zinc in addition to the standard pharmacotherapy regimen in a subgroup of patients with hepatitis C with beta thalassemia major. As was reported by Ko et al., our results suggest that the addition of zinc (30 mg/d) to current standard pharmacotherapy does not significantly improve values of SVR or liver biochemical parameters in newly diagnosed patients with HCV. Our study controlled for parameters such as baseline zinc level, liver biochemical parameters, drug regimen, genotype, stage of fibrosis, and grade of inflammation between the two groups. Unlike the study by Murakami et al.,we did not see any significant difference in ALT level between zinc and placebo group after withdrawal of pharmacotherapy (p=0.616). Murakami et al., used a dose of 150 mg elemental zinc in their study; however, we administered 30 mg elemental zinc to reduce gastrointestinal side effects because many Iranian people cannot tolerate zinc in high doses. This difference in zinc dose could the difference in results in our study versus the Murakami study. It is also possible that zinc administration in patients with thalassemia may be ineffective because zinc may be excreted due to these patients receiving chelating agents such as deferoxamine [12]. These explanations are further supported by the fact that zinc levels were not significantly different before and after treatment in either the zinc group or the placebo group (p= 0.274, p= 0.218, respectively). Although zinc levels increased in the zinc group after beginning the pharmacotherapy (from 0.75±0.19 to 0.84 ± 0.28), statistical analysis showed that this increase was not significant (p =0.274). In a recent study, zinc deficiency has been reported in patients with hepatitis C with or without beta thalassemia major [21]. Although we did not determine zinc depletion in this study, it is recommended that future studies evaluate zinc excretion in both groups.

CONCLUSION

Our data indicate that adding zinc in a dose of 30 mg/d is neither more effective than standard pharmacotherapy nor does it improve serum zinc levels in patients with hepatitis C with beta thalassemia major. It is possible that an increase in the zinc dose 30 mg/d could produce different results. In future studies, we recommend trying higher doses zinc in patients with both hepatitis C and beta thalassemia.

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PARTICULARS OF CONTRIBUTORS:
1.   Faculty, Department of Clinical Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2.   Faculty, Baqiyatallah Research Center for Gastroenterology and Liver Disease, Baqiyatallah University of Medical Sciences, Tehran, Iran; Middle East Liver Disease,Tehran, Iran.
3.   Faculty, Baqiyatallah Research Center for Gastroenterology and Liver Disease, Baqiyatallah University of Medical Sciences, Tehran, Iran.
4.   Faculty, Students Research Committee, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
5.   Faculty, Baqiyatallah Research Center for Gastroenterology and Liver Disease, Baqiyatallah University of Medical Sciences, Tehran, Iran.
6.   Faculty, Baqiyatallah Research Center for Gastroenterology and Liver Disease, Baqiyatallah University of Medical Sciences, Tehran, Iran.
7.   Faculty, Baqiyatallah Research Center for Gastroenterology and Liver Disease, Baqiyatallah University of Medical Sciences, Tehran, Iran.
8.   Faculty, Iranian Blood Transfusion Organization Reaserch Center, Tehran, Iran.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Mohammad Abbasinazari,
Valiasr Street, Nyayesh Junction , School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Phone : +0098 21 9123431245, E-mail : m_abbasi@sbmu.ac.ir

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