Therapeutic effects of deferoxamine and silymarin versus deferoxamine alone in β-thalassemia major based on findings of liver MRI

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BACKGROUND: Thalassemia is an inherited disorder in which repeated blood transfusion is needed. This causes accumulation of iron in various organs. Silymarin is a natural medicinal plant that has been used for centuries in the treatment of liver depositions. In this study, magnetic resonance imaging (MRI) of liver, which has high accuracy in measuring liver iron concentration (LIC), was used to evaluate the effects of silymarin (Legalon) as an iron-chelating agent. **METHODS:** In this double-blind randomized clinical-trial study, 48 thalassemic patients were enrolled during a period of 6 months. In order to evaluate the efficacy of Legalon on LIC, the control group was treated with deferoxamine and placebo tablets while the case group received a combination of deferoxamine and silymarin. MRI was performed for all patients before and after the intervention to determine liver iron using Gandon's protocol. **RESULTS:** Overall, 37 patients, including 22 controls and 15 cases, completed the study. The mean concentrations of liver iron at baseline were 290 ± 62 μmol/g and 328 ± 27.8 μmol/g in the control and case groups, respectively. The mean liver iron concentrations after 6 months of intervention were 290.4 ± 65.4 μmol/g and 334.6 ± 27.9 μmol/g in the control and case groups, respectively. No group experienced a significant difference in LIC change before and after the 6-month trial ($p = 0.43$). **CONCLUSIONS:** Administration of silymarin did not cause significant changes in liver iron concentration. Evaluating a longer course of treatment with this drug is thus suggested.
KEYWORDS: Beta-Thalassemia; Magnetic Resonance Imaging; Silymarin, Deferoxamine.

BACKGROUND

Thalassemia is the most common genetic disorder in the world and nearly 200 million people are affected by this disease. Beta-thalassemia (β- thalassemia) is seen in 10-15% of the population in Mediterranean and south eastern Asia.[1]

β-thalassemia occurs due to deficiency in the production of β-globin which in turn causes a relative increase in alpha-globin. Extended alpha-globin is unstable and they can't make the solution tetramer. This leads to a number of symptoms whose severity is determined by the extended chain. In homozygote cases, there is a severe deficiency in the production of β-chain and this condition is named as major β-thalassemia. Symptoms begin from the 7th month of life when the production of gamma-chain decreases and the production of mature β-chain in hemoglobin is increased instead.[2-6] The initial symptoms include pale face, irritability, delayed growth, and abdomen expansion due to hepatosplenomegaly. These symptoms are all complications of hemolytic anemia.

In 80% of untreated children, death occurs in the first 5 years of life because of severe anemia, heart failure, and infections.[7,8]

Major β-thalassemia involves different organs. Skeletal changes are significant and the disease causes delayed skeletal growth and significant changes in the face and body.[9]

Hepatomegaly appears at the primary stages of β-thalassemia and is caused by increased red blood cell destruction and extra medullary hematopoiesis in the liver. In developed stages, hypoalbuminemia, abnormality of blood coagulation factors, and other symptoms of severe liver disease are caused by hemochromatosis. The patient is thus exposed to hepatitis B virus (HBV) and hepatitis C virus (HCV) during recurrent injections.[10]

Some of other complications are biliary stone, huge splenomegaly which always needs splenectomy, renal enlargement due to extra medullary hematopoie sis, endocrine and metabolic abnormalities, delay in

primary and secondary sexual characteristics in both sexes, and cardiac abnormalities.[11]

Treatment of β-thalassemia is based on chronic blood transfusion, splenectomy, iron chelation drugs, and supportive measures.[12]

Widespread repeating of blood transfusion changes the clinical course of disease in the world. Among the causes of patient death are the side effects of oxidative stress due to iron load. Such complications include disorders of the immune system and infections caused by blood transfusion.[12-14] Therefore, the most important problem in these patients is the accumulation of iron in the body mostly in the liver, hypophysis, pancreas, and heart which causes peroxidative damage to these organs.[15]

Repeated blood transfusion leads to iron accumulation. Iron chelating factors are effective in prevention from iron toxicity and oxidative stress. The only effective and accessible drug for iron load treatment due to repeated blood transfusion and red blood cells hemolysis in these patients is desferrioxamine. Although it had been widely used in treatment of β-thalassemia, its expensiveness, difficulty of use (since it can only be employed through injection), and visual and auditory side effects limited its application.[16] These factors necessitated a new drug in a more simple oral form.

Silymarin is taken from milk thistle (silybum marianum). It acts as an antioxidant and has been used in liver diseases for centuries. It controls lipid peroxidation, increases hepatocyte protein synthesis especially glutathione, and decreases the activity of carcinogens, mast cells stabilizers, and chelate iron.[17,18] In addition to the role of silymarin as an antioxidant and iron toxicity reducer, its role in liver cirrhosis was shown in rats.[19] While silymarin is probably a natural multifunctional drug, its therapeutic effects have not been yet clearly identified.[20] However, the therapeutic effects of silymarin in major β-thalassemia have been first evaluated in Iranian in-vitro and in-vivo studies.[21] The results of in-vitro examinations on thalassemia patients showed that silymarin led to increased glutamine level and proliferation of mono nucleus cells in peripheral blood.[21] In-vivo studies showed combined treatment with silymarin and deferoxamine to significantly improve liver functional tests compared to receiving deferoxamine alone.[22]

Although these findings suggest the probable role of silymarin in treatment of iron concentration among β-thalassemia patients, they should be approved by exact methods to evaluate iron supply. Ferritin is a molecule that supplies iron in human body. Plasma ferritin concentration is an indicator of the amount of ferritin in body cells. It is also used as a diagnostic indicator for the liver iron supply.[23] In fact, serum ferritin concentration could be increased during inflammation, infection, malignancies, hepatic and some other diseases which could be seducer.[24]

Magnetic resonance imaging (MRI) is currently known as a suitable and non-invasive method to evaluate liver iron content (LIC). While various protocols are available for MRI, Gandon et al. proposed a new technique in 2004. Their results were proved based on liver biopsy .[25-27]

The aim of this study was to use the method of Gandon et al. to evaluate LIC in patients with thalassemia before and after our intervention. The role of silymarin in decreasing iron viscosity in liver in major β-thalassemia was determined.

METHODS

This double-blind randomized clinical trial study was performed in 2010. Patients were randomly selected from the thalassemia clinic of Seyed Al Shohada Hospital in Isfahan, Iran. This project was approved by Isfahan University of Medical Sciences (research project number: 187050).

From β-thalassemia patients, 48 volunteers were enrolled in this study and after describing the aim of the study, they signed an informed consent in order to participate in the study.

The inclusion criteria were having major β-thalassemia, being older than 12 years old (because of lacking studies for silymarin doses in younger cases), serum blood ferritin between 1000-5000 mg/dl, being under regular treatment by deferoxamine (40 mg/kg), undergoing regular blood transfusion, having hemoglobin levels equal to or higher than 9.5 mg/dl by repeated blood transfusion, and negative C-reactive protein (CRP) at the beginning of the study.[28,29]

On the other hand, the exclusion criteria were having HCV, HBV, or human immunodeficiency virus (HIV) infection, cardiac or renal failure, digestive problems that prevented silymarin absorption, pregnancy,[30] using other drugs except deferoxamine as iron chelator, and MRI preventive factors (claustrophobia or having a metal device in the body such as heart artificial valve.

During the study, patients were excluded if symptoms

of sensitivity to silymarin or intolerance appeared, or another oral chelator drug such as L1 was started.

In this study we compared the efficacy of adding either Legalon (a product which contains silymarin) or placebo tablets to the routine iron chelating agents in improving LIC of β-thalassemic patients.

We divided the patients into two groups by simple random allocation. The first group continued to use deferoxamine with placebo tablets (control group) while the second group used a combination of deferoxamine and silymarin product (Legalon) (case group).

Active Legalon tablets contained 80% silymarin (Madaus Pharma, Germany). Placebo tablets were similar to drug tablets in shape, but did not contain the active component.

Although silymarin (Legalon) dosage varied between 240 and 800 mg/day in previous studies, the common dose as a helping treatment has been one 140 mg tablet 3 times daily.[32-34] Therefore, in this study, silymarin was used in the form of 140 mg Legalon tablets, one hour before each meal (3 times daily) for 6 months. The control group used placebo tablets with the same protocol.

Liver MRI was performed before and six months after the intervention for all participants in Alzahra Hospital, Isfahan, Iran. A 1.5-Tesla MRI system (Siemens, Germany) was used in all evaluations.

LIC was assessed by measuring the ratio of liver signal to the adjacent muscles. In each patient 5 sequences were performed, i.e. gradient echo (GRE) T1 and T2 (GRET1 and GRET2), GRE proton density (GREPD), GRET2+, and GRET++. The severity signal in each sequence was calculated considering 5 areas of 1 cm^2 as region of interest (ROI) for each sequence. Overall 3 ROI of right liver lobe and two ROI in right and left paraspinal areas were considered. The relative signal of the liver to paraspinal muscles was calculated. In order to calculate LIC, the data was entered into the software proposed by Gandon which is accessible at http://www.radio.univ-rennes1.fr/Sources/EN/HemoCalc05.html.[31]

LIC was measured before and after the medical intervention in both groups. Data was analyzed by chi-square test to compare the quantitative variables. Since variance distribution of LIC before and after the inter-vention (LIC1 and LIC2, respectively) in each patient did not follow a normal distribution (using Kolmogorov Smirnov test, p = 0.01), Mann-Whitney test was used to examine the effects of the drug on LIC variations of the patients.

RESULTS

From 48 patients, 37 cases completed the study. The other 11 cases left the study due to immigration (1 case), refusing to continue participation (1 case), non-compliance to taking the drug properly (4 cases), and need for administration of another drug as a result of other thalassemic complications (5 cases). None of the cases encountered drug intolerance or side effects.

From these 37 cases, 27 cases were in the control group and 15 were in the case group. Mean ages in the control and case groups were 18.3 ± 5.9 and 20.1 ± 4.1 years, respectively (p > 0.05). Females constituted 53.3% of the case group and 47.1% of the control group (p > 0.05).

Mean LIC1 was 290.0 ± 62.0 µmol/g in the control group and 328 ±27.8 µmol/g in the case group. Therefore, the 2 groups were significantly different at baseline (p = 0.02) (Figure 1).
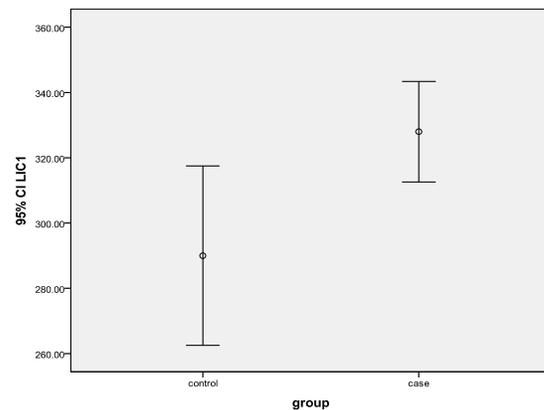

Figure 1. Liver iron content at the beginning of the study (LIC1) in the case and control groups (reported by 95% confidence interval)

Mean LIC2 was 334.6 ± 27.9 µmol/g in the case group and 290.4 ± 65.4 µmol/g in the control group (p = 0.01) (Figure 2).

Mann-Whitney test showed a statistically significant difference in LIC1 between the two groups (p = 0.02). On the other hand, there was not any statistically significant intragroup difference between LIC1 and LIC2 of patients in either group (p = 0.37). Moreover, being a
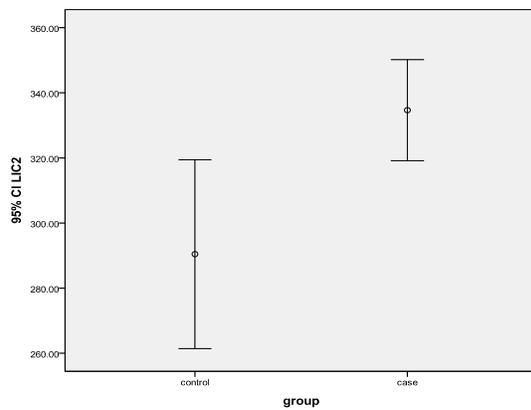
Figure 2. Liver iron content after the intervention (LIC2) in the control and case groups (reported by 95% confidence interval)

member of the control or case group did not make any significant difference in LIC before and 6 months after the treatment (p = 0.43).

The mean difference of LIC before and after the intervention (LIC1-LIC2) was 28.86 ± 0.45 μmol/g in the control group and 11.13 ± 6.67 μmol/g in the case group (p = 0.51).

DISCUSSION

β-thalassemia is a life threatening disease that makes significant impairments in the function of organs due to iron overload. In addition, its various complications can lead to high mortality rates. Therefore, finding new therapeutic modalities for this disease is still a challenging concept in the field of medicine.

As a well-known iron chelating agent, deferoxamine has been widely used to prevent iron overload in β-thalassemic patients for several years. However, it is moderately efficient, the injection is painful, and severe side effects like serum sickness may occur. Finding new therapeutic agents could hence help thalassemic patients by decreasing disease complications and improving quality of life.

Alidoost et al. reported the efficacy of silymarin in treatment of β-thalassemia in Iranian patients. They revealed that taking silymarin in thalassemic patients led to increased amount of glutamine and proliferation of mono nucleus blood cells.[21]

Gharagozloo et al. used a combined treatment containing silymarin and deferoxamine for 3 months. They showed that liver function tests improved significantly compared to the patients who merely received deferoxamine.[22]

In contrast to these studies, our research did not reveale any additional benefit for silymarin over placebo. It could be due to our measuring method (MRI) and limited number of participants.

Increasing silymarin dosage and the duration of drug administration may better decrease iron concentration. Our patients were not matched for LIC at the beginning of the study and it is one of our significant limitations. Considering a more accurate matching in baseline parameters like duration of disease and LIC for better judgmental results should be weighted for future studies. This subject can be a good topic for further research.

In addition, we should mention that the method of Gandon et al. is not accurate enough in determining LICs above 300 μmol/g. As LIC was higher than 300 μmol/g in many of our cases, the selected method might have not been quite accurate.

Using innovative MRI methods with higher accuracy in detecting larger amounts of LIC or administrating concomitant laboratory parameters (such as ferritin) as more definite indicators of iron supply of the body could validate the scientific value of our results and also prevent any additional bias.

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