



Research Article

Protective Effects of Selenium in Patients with Beta-Thalassemia Major

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Abstract

Background: Beta-thalassemia major patients require repeated blood transfusion which is associated with iron overload in different organs such as heart, liver, kidney and their related complications. In this study the effects of selenium in iron overload related complications of patients with beta-thalassemia major were assessed.

Methods: In this clinical trial, 34 beta-thalassemia major patients over 12 years old were enrolled. Patients with severe renal failure, history of selenium consumption over the last three months, change of blood transfusion pattern, and any change of chelating agent were excluded from the study. For all patients, tablet of selenium 200 µg/day was administered for a month. Blood samples were taken at baseline and after one-month to assess the level of ferritin, total iron-binding capacity (TIBC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum creatinine (Scr), selenium. Hair loss was assessed by questionnaire before and after intervention.

Results: From 34 patients, 27 (79.4%) had deficient level of selenium at baseline. The selenium level was increased after intervention ($p=0.005$). The level of serum ALT and Scr decreased remarkably after one-month selenium consumption ($p=0.007$ for both). In addition, the AST level decreased remarkably after intervention ($p=0.053$). Severe hair loss profile has improved significantly after supplementation ($p=0.004$).

Conclusion: One-month selenium consumption improved liver and kidney function related markers remarkably. Moreover, selenium improved hair profile and severe hair loss in thalassemia patients. Further studies are needed on the effect of selenium administration on liver and kidney function.

Introduction

Thalassemia is a category of hematological single gene disorders resulted from defects in the hemoglobin chain production.¹ It is predicted that around 1.5% of the world population are carriers of beta-thalassemia gene.² The overall annual incidence of clinical manifestations of this disorder is estimated at 1 in 100,000.² In beta thalassemia, the production of beta chains has decreased and alpha chain is produced consistently.³ Patients with beta-thalassemia major require frequent blood transfusion which leads to iron overload within body organs.⁴ The long-term clinical presentations of iron overload are heart failure, liver disorders and endocrine abnormalities.¹ In addition, iron overload accounts for increased oxidative stress, free radical synthesis and upcoming oxidative damage, which increases the mentioned complications.⁵ Cirrhosis and hepatocellular carcinoma may develop as a result of chronic hepatitis and/or severe iron overload.⁶ In a retrospective

study by Li *et al.* on 100 thalassemia major patients, the liver biopsy and histological study were performed for all patients to assess their liver iron content and fibrosis or hepatitis, respectively. Moreover, in the two years prior the liver biopsy ALT levels were routinely measured before monthly transfusion. Their results showed that both hepatic fibrosis and hemosiderosis were significantly associated with higher serum ferritin and liver enzymes, particularly alanine aminotransferase (ALT).⁷ Moreover, renal dysfunction happens in thalassaemic patients because of different causes such as renal hyperfiltration, hypercalciuria, and albuminuria. Besides, high transfusion intensity and iron chelators exposure exacerbate renal dysfunction in these patients.^{8,9}

Minerals play a major role in growth, protein synthesis, and many enzymes activation. Because of high oxidative stress existence and iron chelators consumption, patients with

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thalassemia are at increased risk of mineral deficiency.¹⁰ Selenium is a trace element which presents its biological function in form of amino acid selenocysteine (Sec) and its incorporation into proteins.¹¹ Selenoproteins such as glutathione peroxidase plays a critical role in the defense system against oxidative stress by conserving free radical damage.¹¹

Selenium plays an essential role in oxidative damage protection and hair follicle morphogenesis.¹² Previous experimental studies showed that selenium deficiency tender hair growth.¹² Lacking specific selenoproteins showed oncoming hair loss after birth in knockout mice.¹² Although, there is a lack of human studies, most hair loss supplements in markets contain selenium. In addition, due to the patient's statement about the effect of the selenium supplement in hair loss and hair quality, we decided to assess the effects of selenium on hair loss too. subsequently, questionnaire was designed to assess the hair loss.

The aim of this study was to assess the different effects of selenium in related complications of patients with beta thalassemia major.

Materials and Methods

Patient enrollment

This study was conducted under the supervision of general practitioner on Shahid ghazi thalassemia outpatient clinic, Tabriz, Iran, from February till may 2017. This trial was registered in the Iranian Registry of Clinical Trials (Registry number: IRCT2017021226998N3).

After institutional review board approval 34 patients with beta thalassemia major over 12 years old were enrolled in this clinical trial (the G*Power 3.1 was used for sample calculation with the $\alpha=.05$ and the power of 80%). Patients with severe renal failure (GFR<30), any change in blood transfusion pattern, alteration in dose and type of chelating agent, and history of selenium supplementation over the last three months were excluded from the study. Demographic information, co-morbidities, type of chelating therapy were recorded for analysis. During the study, patients' nutritional diet remained intact and assessed by food questionnaire. Information on food intake was collected by using the three dietary record questionnaires for 3 days (including 2 working days and 1 weekend) before and at the end of supplementation.

Intervention

For all patients 200 μ g tablet (daily requirement) of selenium yeast (organic selenium, Webber Naturals) were administered daily for one-month. Fasting blood samples were taken at baseline and after one-month. The level of ferritin, total iron binding capacity (TIBC), serum iron (Fe), serum creatinine (Scr), blood urea nitrogen (BUN), ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), albumin (Alb) and fasting blood sugar (FBS) were measured before and after intervention. In addition, extra one samples were taken and centrifuged

at 3000 rpm for 5 minutes. The clean serum was stored at -22 °C until the time of serum selenium assessment.

Selenium level assessment

The serum selenium concentration assessment was carried out by the atomic absorption method. Atomic absorption spectrophotometer (Model CTA 3000) with deuterium lamp background correction and quartz T atomizer was employed to detect selenium.¹²

In the serum samples selenium can be detected predominantly as selenoproteins. In order to release it from these proteins, wet acid digestion method was conducted.¹³ In a glass beaker, 500 μ L of serum and 10 mL of concentrated nitric acid and 6 mL of hydrogen peroxide were placed under the fume hood. The resultant solution was heated in oil bath at 140°C to dry (4 hours). Then sera with selenium element inside were put in spectrophotometer to be measured by atomic absorption method.¹³

The normal range of selenium concentration in adult serum is 70 to 150 ng/ mL and in the serum concentration below 40 ng/mL the activity of glutathione peroxidase is lost.^{14,15}

Hair loss assessment

Two questions were selected from a questionnaire of Midland Skin Institute (MSI), Vancouver, and were replied by patients at baseline and after intervention. The two enquiries are focused on hair loss and stated as follows: 1) What is your hair loss quality (which assesses hair loss quality)? hair coming out or shedding, hair looked thinner on scalp/both/none; 2) Have your hair loss is getting worse and severe recently (which assesses severe loss)? yes/no.

Statistical analysis

Continuous variables were described as mean and standard deviation (SD) or median with 25th and 75th quartiles whenever variables were not normally distributed. Categorical variables were expressed as frequency and percentage. The normality distribution was assessed by Kolmogorov Smirnov test. The baseline and after one-month data were analyzed using paired t-test and Wilcoxon signed-rank test for data with normal and non-normal distributions, respectively. The relation of quantitative variable was shown with Spearman correlation coefficient. P-values less than or equal to 0.05 were considered statistically significant. Statistical analysis was performed using the IBM SPSS statistics for Windows, version 20.0.

Results

In this clinical trial, 84 patients over 12 years old were assessed for eligibility (the all number of thalassemia patients in our province) from which 40 patients did not meet the inclusion criteria, six refused to participate in the study and two were excluded because of renal failure and change in blood transfusion pattern. Finally, 36 patients were selected. From all, two patients left the study because

of gastrointestinal side effects (Figure 1). Therefore, 34 patients remained and successfully completed the study. No differences were observed in daily dietary intake at baseline and after supplementation. Demographic information, baseline comorbidity and chelating agents are demonstrated in Table 1.

From 34 patients, the level of serum selenium was >70 ng/mL in 7 (20%) patients and >40 ng/mL in 14 (41%) of them. Subsequently, due to the standard values^{14,15} the proportion of selenium deficiency and lack of glutathione peroxidase activity were 79.4% (Figure 2) and 58.8%, respectively, in patients with beta thalassemia major. The level of serum selenium increased from 42 ± 37 to 71.2 ± 52 ng/mL after a month supplementation ($p=0.005$). Our data showed that, the level of serum iron, ferritin, ESR, BUN, LDH and FBS decreased after intervention (Table 2). In addition, as illustrated in Table 2 the serum level of ALT and Scr improved remarkably after supplementation ($p=0.007$ for both). Although the AST level decreased remarkably, it was not statistically significant ($p=0.053$).

It is noticeable that severe hair loss has decreased dramatically ($p=0.004$) without any changes in hair loss quality ($p=0.504$) after intervention. Women were more susceptible to hair loss and improved hair loss profile obvious than men (Table 3).

Hypothyroidism showed no effect on hair loss quality at baseline ($p=0.255$) and after intervention ($p=0.187$). Furthermore, there was no relation between severe loss and hypothyroidism ($p=0.064$). In addition, the effect of baseline selenium concentration on hair loss quality and severe loss was not significant. All data were adjusted with respect to the type of chelating agents and the results did not change statistically.

Discussion

Selenium deficiency

In this study the mean serum selenium concentration was in deficient level at baseline and the incidence was 79.4%. However, after a month supplementation the serum level of selenium was increased.

The main reasons for selenium deficiency in normal population is the absence of dietary diversity and low

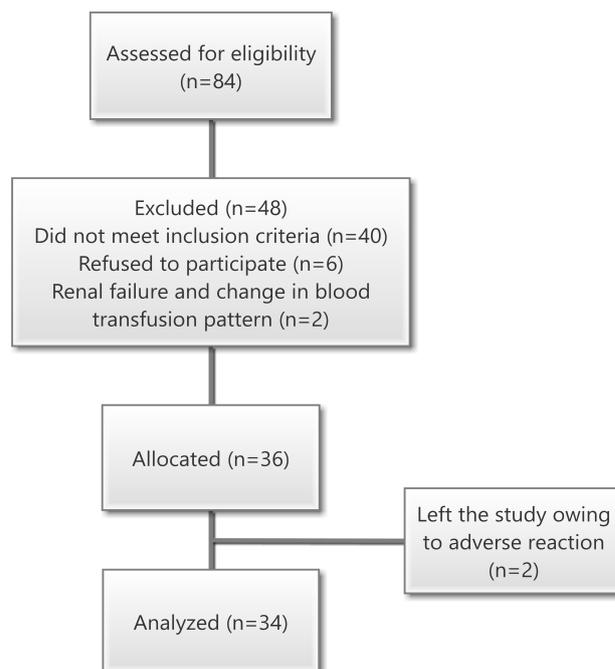


Figure 1. Screening and follow up of patients.

Table 1. Demographic information and baseline characteristics of patients with beta thalassemia major.

| Characteristics | | Mean \pm SD/ n (%) |
|-----------------|-------------------------|----------------------|
| Age | | 21.4 \pm 4.5 |
| BMI | | 21 \pm 2.2 |
| Gender | Female | 9 (26.5) |
| | Male | 25 (73.5) |
| Comorbidity | None | 27 (79.4) |
| | Hypothyroidism | 1 (2.9) |
| | Diabetes | 2 (5.9) |
| | Diabetes+Heart Failure | 2 (5.9) |
| | Diabetes+Hypothyroidism | 1 (2.9) |
| | Fatty liver | 1 (2.9) |
| Chelating agent | Desferal | 11 (32.4) |
| | Deferasirox | 1 (2.9) |
| | Desferal+Deferepirone | 21 (61.8) |
| | Desferal+Deferasirox | 1 (2.9) |

Continuous variables are described in mean \pm standard deviation (SD). Categorical variables are presented in percent (%). BMI: body mass index.

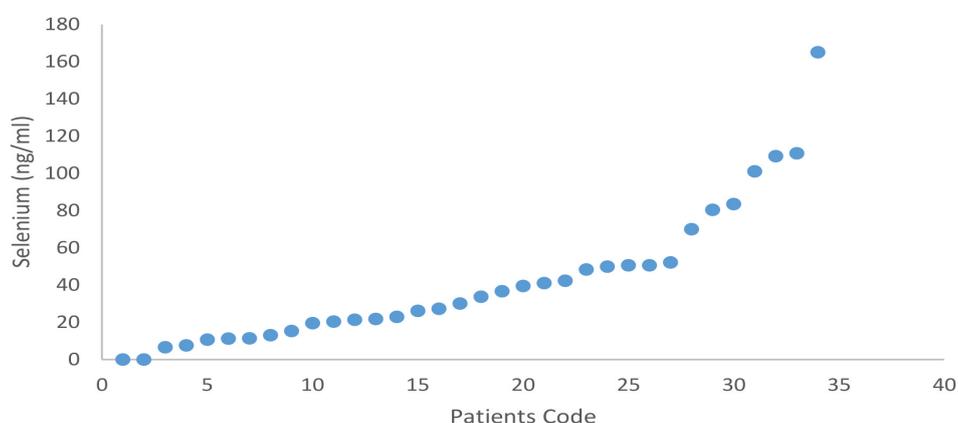


Figure 2. Baseline serum selenium concentration of patients.

Table 2. Comparison of baseline and after intervention lab data of patients with beta thalassemia major.

| Variable | Baseline Mean±SD/Median(IQR) | After intervention (Mean±SD)/Median(IQR) | p-value* |
|--------------------------|---------------------------------|---|----------|
| Serum iron (µg/dL) | 214.6±71.9 | 202.1±55.4 | 0.09 |
| Ferritin (ng/mL) | 5122.5±2936 | 5010.3±2959.2 | 0.74 |
| ESR (mm) | 16±18.5 | 14.6±12.6 | 0.55 |
| BUN (mg/dL) | 28.9±6.1 | 27.2±8.1 | 0.26 |
| ALP (IU/L) | 390±194 | 418.6±213.9 | 0.34 |
| Selenium (ng/mL) | 42±37.1 | 71.2±52.5 | 0.005 |
| Serum creatinine (mg/dL) | 0.52(0.1) | 0.44(0.1) | 0.007 |
| ALT (IU/L) | 44 (26.9) | 35.1(20.6) | 0.007 |
| AST (IU/L) | 41.47(17.56) | 36.82(17.47) | 0.059 |
| TIBC (µg/dL) | 239.5(74.25) | 248(64.5) | 0.453 |
| LDH (IU/L) | 411(157.2) | 409(166) | 0.080 |
| FBS (mg/dL) | 137.5(101) | 121.1(67.4) | 0.84 |

Normally distributed variables are presented as mean ± standard deviation (SD). Non-normally distributed variables are presented as median and interquartile range (IQR).

*Paired T test is used to evaluate p values of normal parameters and Wilcoxon Signed Ranks test is used to determine non-normal parameters.

ESR: erythrocyte sedimentation rate; BUN: Blood urea nitrogen; ALP: Alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TIBC: Total iron-binding capacity; LDH: Lactate dehydrogenase; FBS: fasting blood sugar.

Table 3. Hair loss data in patients with beta thalassemia major.

| Parameter | Degree | Female n (%) | Male n (%) | P-value* |
|---|---------|-----------------|---------------|----------|
| Hair loss quality (baseline) | Loss | 5 (55.6) | 3 (12) | 0.03 |
| | Thinner | 1 (11.1) | 4 (16) | |
| | Both | 1 (11.1) | 1 (4) | |
| | None | 2 (22.2) | 17 (68) | |
| Hair loss quality (after intervention) | Loss | 6 (66.7) | 3 (12) | 0.006 |
| | Thinner | 1 (11.1) | 1 (4) | |
| | Both | 0 (0.00) | 4 (16) | |
| | None | 2 (22.2) | 17(68) | |
| Severe hair loss (baseline) | Yes | 5 (55.6) | 4 (16) | 0.03 |
| | No | 4 (44.4) | 21(84) | |
| Severe hair loss (after intervention) | Yes | 0 (0.00) | 0 (0.00) | - ** |
| | No | 9 (100) | 25 (100) | |

*Fisher's Exact test is applied to compute P-value for association between gender and hair loss parameters

**Cannot be computed since severe loss (after study) is constant

selenium concentrations of soil.¹⁶ Serum selenium concentration in normal populations is different from 41.7 µg/L to 158.2 µg/L in different countries.¹⁷ The mean serum selenium level of normal adults was 100.6 ± 12.8 µg/L in Tehran, Iran.¹⁸ Mashhadi *et al.* showed that serum selenium concentration was in deficient level in 25.52% of thalassemia patients in south of Iran.¹⁹ Previous studies demonstrated that the mean serum selenium level was 31.5±19.5 µg/L and 44.87 ± 9.84 µg/L in Egyptian and Iraqi major thalassemia patients, respectively.^{20,21} The serum selenium level was deficient in 75% of patients with thalassemia major in California.^{22,23} The frequency of selenium deficiency in our study is similar with most of the previous studies (42±37.1 µg/L).

Although the serum selenium concentration strongly depends on selenium concentration of soil in normal population, in thalassemia patients it depends on multiple factors. It is obvious that the basic origin of dietary selenium is protein.²⁴ Thalassemia patients have lower protein intake that can be one of the causes of selenium

deficiency in this population. Moreover, previous studies showed that the gastrointestinal absorption of selenium is low in iron overloaded thalassemia patients.²⁵ In addition, iron chelators consumption may increase different mineral deficiencies in this population.²⁶

Because of repeated blood transfusion and iron accumulation, oxidative stress has increased in thalassemia patients. In iron overload patients, iron stored in hepatocytes after transferrin saturation. When hepatocytes are overloaded, non-transferrin bound iron (NTBI) released into circulation and deposited in organs such as hepatocytes, myocytes, pituitary and pancreatic cells.²⁵ NTBI metabolism leads to the reactive oxygen species (ROS) generation such as hydroxyl radicals and superoxide anions which causes cellular damage.²⁶ Selenoproteins such as glutathione peroxidase and thioredoxin reductase, lead to termination of ROS activities by catalyzing hydrogen peroxide (H₂O₂) or organic hydroperoxides to water or alcohols by using glutathione (GSH) as reductant.^{11,27} GSH is a thiol-containing peptide which exists in variety of cells such as liver, kidneys, spleen and erythrocytes. Due to the strong potential of electron-donation, GSH is the critical peptide for cellular redox maintenance.²⁸

Glutathione peroxidase activity depends on selenium level, antioxidant consumption, oxidative stress existence and duration. Glutathione peroxidase activity decreased in deficient level of selenium because of the role of this element in its structure as a selenoprotein.¹¹

Glutathione peroxidase needs GSH as a reductant and selenocysteine as the catalytic section to start its activity.²⁷ In thalassemia patients the pool of intracellular GSH is depleted because of iron accumulation mediated oxidative stress.²⁸

Hepatoprotection

ALT and AST are indicators of liver function and when their level are above the normal range, it represents liver

injury. It is highly likely that these patients suffer from iron deposition in liver and prone to develop liver damage. Hence, enzymatic markers like ALT and AST tend to be higher in patients with thalassemia. In this study selenium supplementation decreased the elevated serum levels of ALT ($p=0.007$).

The liver disorder is the important reason of mortality in thalassemia patients. NTBI is highly toxic and deposits in the hepatocytes. NTBI generates the production of free radicals, which have been involved in lipid peroxidation. Lipid peroxidation is the known cause of hepatocellular injury induced by iron overload. Severe iron overload leads to cirrhosis and hepatocellular carcinoma.²⁹ Li *et al.* demonstrated that hepatic fibrosis and hemosiderosis were remarkably associated with high serum ferritin and alanine aminotransferase (ALT).⁷ The liver has essential role in the metabolism of selenium within the body since most of the selenoproteins are produced and released by the liver.^{30,31} Hepatic impairment causes derangement of selenoproteins synthesis, thus leading to low serum selenium level. In an experimental study Czuczejko *et al.* showed that selenium deficiency induced liver necrosis.²⁴ Furthermore, the autopsy of patients with cirrhosis of the liver proved a remarkably lower selenium content compared to healthy ones. In addition, Czuczejko *et al.* demonstrated that the whole blood and serum selenium concentration was low in patients with any kind of liver disease with different reasons such as viral hepatitis, alcoholics, auto-immune and cryptogenic chronic liver disease patients.²⁴ Moreover, the level of GSH is decreased in patients with liver diseases as a reason of decreased inflow from the hepatocytes. Above-mentioned findings indicate that impairment in antioxidant levels may generate ROS production which play a role in the pathogenesis of hepatic dysfunction. Selenium supplementation may improve glutathione peroxidase activity in order to reduce oxidative stress in hepatocytes and can reduce liver injury in thalassemia patients. In this study the serum selenium concentration was assessed instead of tissue concentrations. It can be seen that because of high oxidative stress in thalassemia patients, cells are depleted from selenium and related proteins. Subsequently, the association between baseline organ dysfunction and serum level of selenium does not ignore the hepatocellular protection of selenium in this study.

Renoprotection

From the all organ disorders, renal dysfunction is less known in thalassemia patients. Variety of abnormalities including renal tubular dysfunction, increased renal plasma flow, decreased ability of urine concentrating, and renal tubular acidosis are reported in thalassemia patients.⁸ Our data showed that one-month selenium supplementation reduced Scr significantly in thalassemia patients. Iron overload toxicity and anemia are the main reasons of mentioned abnormalities. Previous studies showed that these abnormalities were related to the duration of

chelation and transfusions, content of transfused iron, and amount of body iron.³²⁻³⁶ Existence of chronic hypoxia and anemia are related with increased lipid peroxidation, oxidative stress, and functional impairment in tubular cells.⁹ In addition, treatment with chelating agents such as deferoxamine and deferasirox affect renal function in patients with thalassemia.⁸ In proximal tubule fluid the iron released from transferrin in acidic environment. Produced free reactive iron stimulates the generation of ROS and cellular damage.⁹

Different studies showed beneficial renoprotection effects of selenium in lead and cadmium toxicities.^{37,38} Plasma glutathione peroxidase produced mainly by kidneys.²⁴ Selenium can increase glutathione peroxidase production and activity. The increases in glutathione peroxidase activities decline free radicals which are mediated by lipid peroxidation in iron overload status and regenerate GSH. Subsequently, selenium supplementation in an optimum dosage can reduce renal dysfunction in thalassemia patients.

Hair loss

Although the purpose of this study was to evaluate the liver and renal function, patients who received selenium claimed dramatic hair loss improvement during supplementation. Thus, questionnaire was designed to assess the hair loss. Severe hair loss decreased dramatically after one-month selenium supplementation. This effect obvious was especially in women.

Although selenium deficiency results in various organs dysfunction, hair loss is often related with excess amount of oral selenium intake.³⁹ In a case series of four children who were on long-term total parenteral nutrition, selenium deficiency happened and caused hair hypopigmentation in two.⁴⁰ Selenium deficiency was associated with hair loss after cisplatin-containing chemotherapy regimen administration, which was responded to selenium supplementation.^{41,42} The role of oxidative stress on hair condition was increased with impression on pre and post-emerging fibre parameters.⁴³ Sengupta *et al.* showed that the selenoproteins deficiency in skin epidermal cells causes impairment in epidermal cells differentiation and improper morphogenesis of hair follicle and hair formation, subsequently causing oncoming hair loss.⁴⁴ Shaft formation impairment, hair follicle appearance changes, early impaired follicles regression with subcutaneous fat reduction were seen in mice with selenoproteins deficiency.⁴⁴ It is seen that the effect of selenium on hair loss is related with selenoproteins activity against oxidative stress in thalassemia patients.

To the best of our knowledge, the incidence of hair loss and hair problems are not investigated in thalassemia patients. In addition, the effect of selenium in hair loss is not obvious even in normal population. Our observation and statistical data showed a dramatic and significant hair loss improvement in thalassemia patients particularly in women. Because of the limited number of patients in

our center, the randomized clinical trial did not perform. The serum level of selenium was assessed instead of selenoprotein P which most reflect the stock selenium. The duration of supplementation was short (one-month). The cardiac and liver magnetic resonance imaging (MRI) findings were not assessed before and after intervention. The analysis of hair loss was self-reported, not quantified by standardized analyses. In addition, assessment of hair loss was not done by an independent observer. However, in this study we noticed that standard supplementation with selenium can improve thalassemia patients' complications. In addition, the hair problem is the neglected complication in these patients and it may be the reason of inappropriate supplementation such as selenium.

Conclusion

In conclusion, daily selenium (200 µg) supplementation for one-month significantly improved liver and kidney function by the reduction of ALT and Scr. Assessment of liver function with MRI findings before and after intervention is suggested. In addition, selenium prevented severe hair loss and improved hair profile in beta thalassemia major. The protective effects of selenium were independent of baseline selenium concentration. Further studies about the protective effects of selenium in cardiac, liver, kidney function and hair problems of thalassemia patients are needed to design.

Ethical issues

The study protocol was approved by the research ethics committee at Tabriz University of Medical Sciences with IR.TBZMED.REC.1396.13 ethic code. All patients were informed about the study and gave a written informed consent before the study initiation. Patients were free to stay or quit the study at any time.

Data Sharing

Applicants can obtain data by contacting the corresponding author.

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Conflict of Interest

The authors claim that there is no conflict of interest.

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