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*Research Article*

# Effects of Green Tea on Serum Iron Parameters and Antioxidant Status in Patients with β–Thalassemia Major

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**ABSTRACT**

***Background:*** Iron overload and accelerated oxidative stress are main factors in the pathogenesis of β–thalassemia major. The objectives of this study were to examine the effects of green tea consumption on serum iron, ferritin and transferrin saturation (TS) and antioxidant status in β–thalassemia patients.
***Methods:*** This study conducted on 52 subjects with β–thalassemia major (males and females) ≥18 y. The intervention group (n= 26) consumed green tea (2.5 g /150 mL hot water) 3 times per day for 8 wk and the control group (n= 26) followed a water regimen. Anthropometric measurements, blood samples and 24-h dietary recalls were gathered at the baseline and at the end of the trial. Independent t test, paired t test and analysis of covariance were used for data analysis.
***Results:*** Mean of serum iron, ferritin, malondialdehyde (MDA) and total antioxidant capacity (TAC) were 234.7±49.3 µg/dL, 2776.07±533.4 ng/dl, 3.01±0.64 nmol/ml and 2.2665±0.77 nmol/l in green tea group and 298.19±66.87 µg/dL, 3070.23±643.6 ng/dl, 298.19±66.87 nmol/ml and 2.0862 ±0.35 nmol/l in control group at baseline, respectively. Green tea significantly decreased serum levels of iron, ferritin and MDA and increased TAC compared with control group (all, $P < 0.05$). No significant changes were seen in TS value in both groups.
***Conclusions:*** Green tea consumption had favorable effects on iron status and oxidative stress in studied subjects and may be useful in management of these risk factors in patients with β–thalassemia major.

## Introduction

*β*-Thalassemia major is an inherited chronic hemolytic anemia due to impaired globin chain synthesis. Impaired globin biosynthesis results in increasing of unpaired globin chains in erythroblasts and this leads to ineffectual erythropoiesis.[1]

Approximately 1.5% of people in worldwide are affected by this genetic disorder.[2] It is most commonly found in people of Mediterranean descent, such as Italians and Greeks, Middle East and Southeast Asia.[2] In the eastern Mediterranean sections, Iran is one of the main centers for the prevalence of *β*-thalassemia. It is expected that there are between two and three million *β*-thalassemia carriers and 25,000 patients in Iran.[3]

The signs and symptoms of this disease are cardiomegaly, enlargement of bone marrow, severe anemia and splenomegaly. Also impaired erythropoiesis, hemolysis and accumulation of iron in the tissues are some of the clinical manifestations.[4] These patients require periodic or regular transfusions for survival.[5] Moreover, the repeated blood transfusions that *β*-thalassemia patients undergo cause severe iron overload.[6] This secondary iron overload results in excessive generation of free radicals which are capable of causing oxidative impairment to erythrocytes and

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other tissues.[7] Oxidative stress and a possible consequential enhanced apoptosis may contribute to reduced life span of erythrocytes.[4,8]

A suitable iron chelation can result in a net negative iron balance, reduced incidence of hepatic fibrosis, a reduction of cardiac mortality and noticeable improvement in survival and quality of life in this patients.[9-11] Nowadays, drugs such as desferrioxamine, deferiprone and deferasirox with have iron chelators effects, are used for treatment of *β*- thalassemia patients. Unfortunately, most of these chelators have noticeable side effects.[12-15] New researches are now focused toward finding naturally occurring iron chelators of plant origin.[16]

Tea considered as the one of the common beverages consumed worldwide. Tea, from the plant Comellia Sinensis, is consumed in different part of the world as green, black and oolong tea.[17-19] Newly high interest has arisen concerning the health promoting potentials of green tea, in particular as an antioxidant agent.[20,21] The chief components exist in green tea are polysaccharides, vitamins B, C, E, γ-aminobutyric acid, fluoride and polyphenols. Catechins are the most polyphenols in green tea. The main catechins in the green tea are epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG).[22] These polyphenols protect the red blood cell cytosol and membrane from reactive oxygen radicals by strong scavenging property.[23] In addition, these polyphenols are being offered as useful clinical iron chelators.[24,25] Their metal-binding activity suggests that they could be protective agents in pathological disorders caused by oxidative stress in iron overload conditions with a high degree of safety.[26,27] In the study by S. Ounjaijean et al. orally administered green tea extract with a high content of the EGCG (50%) for 4 months decreased iron and lipid peroxidation in plasma and destroyed formation of erythrocyte reactive oxygen species (ROS) in the rats challenged with iron loaded.[28] Saewong et al. demonstrated that orally given green tea extract (300 mg/kg) for 8 weeks inhibits or delays the deposition of hepatic iron and declines liver lipid peroxidation products such as malondialdehyde (MDA) in regularly iron-loaded thalassemic mice.[29] Badiee MS et al. reported that consumption of green tea (12gr/day) by *β*- thalassemia major patients with aged 9-15 y for one month reduced serum levels of iron, ferritin and lipid peroxidation.[30]

Although antioxidant properties and iron chelation effect of green tea have been recognized in vitro and in experimental models of *β*- thalassemia, meanwhile controlled clinical trial about its possible effects on hematological or biochemical conditions of patients with *β*- thalassemia major is rare. Therefore, we initiated a study to examine the effects of green tea consumption on some parameters of iron status (serum iron, ferritin and transferrin saturation (TS) and antioxidant status (MDA and total anti-oxidant capacity (TAC) in patients with *β*- thalassemia major.

## Materials and Methods

### *Subjects*

Fifty-two patients with β-thalassemia major (male & female) aged ≥ 18 y were recruited for this study from the oncologic and hematologic clinic, Qazi Tabatabaei Hospital in Tabriz, Iran from November 2014 to February 2015. All patients were characterized for having beta globin gene mutation. Patients were eligible for inclusion in the study if they were diagnosed with β-thalassemia major and were regularly transfused to maintain the hemoglobin level above 9.5 g/dL and received subcutaneous desferrioxamine as an iron chelator. The patient's demographic information data included history of treatment, blood transfusion, concurrent illness, and current medication. Patients were excluded from this trial if they had one or more of the following conditions: hepatitis B or C infection, a history of a positive HIV test, chronic renal or heart failure, iron chelation therapy with iron chelators other than desferrioxamine or pregnancy. All the patients were examined regularly once or twice a month by clinicians. The β-thalassemia major patients underwent regular blood transfusions (one or two units of packed red blood cell every 3–4 weeks) and desferoxamine subcutaneous infusion (20–40 mg/ kg) according to the iron status.

### *Study design*

The study consisted of a single-blinded randomized, controlled clinical trial with treatment and control groups running in parallel for a period of 8wk. Ethical Committee of Tabriz University of Medical Sciences approved the study protocol, and was registered on the Iranian Registry of Clinical Trials website (IRCT ID:IRCT201403303664 N10). All subjects gave written informed consent before clinical trial enrolment. The sample size was determined based on the primary information obtained from the study by Kassab-Chekir for iron stores.[36] Considering 95% confidence interval and 80% power, in 8 week as primary outcome of the study, the sample size was computed to be 22 per group. This number was increased to 26 per group to accommodate the anticipated dropout rate. The participants were randomly allocated in two groups using a block randomization procedure (of size 4) with matched subjects in each block based on sex, age, and body mass index (BMI) (Figure 1). The random sequence was generated using random allocation software by the statistician for the study. The oncologist randomly assigned participants to

an intervention or control group. Whereas patients and the oncologist allocated to the intervention group were aware of the allocated group, outcome assessors and the statistician were blinded to the allocation. At the beginning of the study a general questionnaire was completed for each subject.

### *Anthropometrics and dietary intake assessments*

Body weight was measured using a scale (Seca, Hamburg, Germany), to the nearest 100 gr accuracy without shoes and wearing light clothing. Height was measured using a mounted tape without shoes to the nearest 0.1 cm accuracy. BMI was calculated as the weight in kilogram divided by the height in meters squared. Information about daily dietary intakes was obtained by 24- h recall method for 3 d, including 2 d during the week and 1 during the weekend. A three day average for energy and nutrients intakes of all subjects was analyzed by Nutritionist 4 software (First Databank Inc., San Bruno, CA).

### *Preparation of green tea and intake*

Green tea (Comellia Sinensis) was obtained from the Iranian Institute of medicinal plants, Karaj Iran. The tea bag, containing approximately 2.5 g of green tea, was manufactured on November 2014. These tea bags are a commercially available product. The intervention group (n=26) consumed one cup of green tea infusate (1 green tea bag infused for 12 min in 150 mL hot water without milk or sugar) three times a day immediately after meals (breakfast, lunch, and dinner) for 8 week. The control group (n=26) consumed an equivalent volume of warm water during the 8week period. Subjects were asked to keep a record of all beverages consumed during the clinical trial and maintain their usual dietary intake and physical activity. The compliance of the volunteers for the study protocol was monitored with telephone interviews once a week and counting returned tea bags in person every 2 wk.

### *Blood sampling and biochemical assays*

Venous blood samples was taken from each subject after a 12-hour fasting at 8-10 am, just before they received transfusion at beginning and end of the study. The serum samples were separated from whole blood by centrifugation at 3500 rpm for 10 min (Avanti J-25, Beckman, Brea, CA, USA). The serum samples were frozen immediately at -70$^{0}$ $^{C}$ until assay. Serum iron was measured using the colorimetric methods with commercially available Pars Azmun kit (Karaj, Iran).

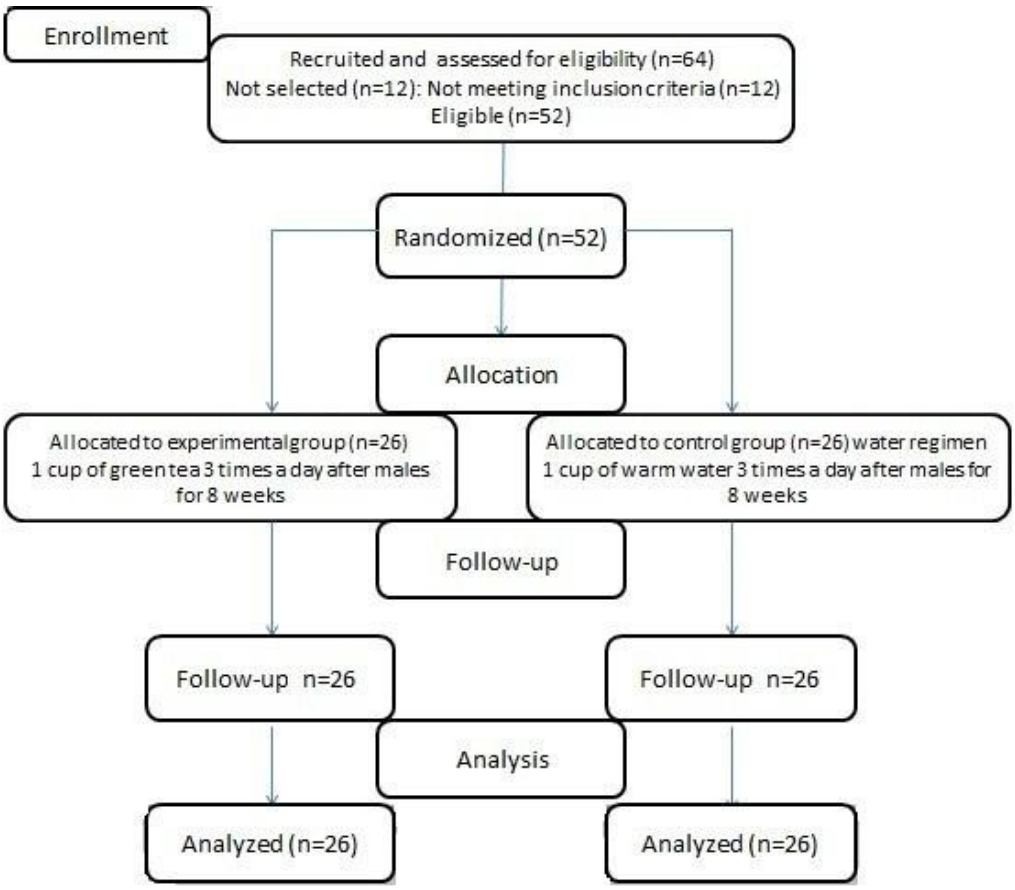


**Figure 1**. Flow diagram showing trial profile and study design.

The amounts of ferritin in serum samples were determined using a ferritin ELISA kit (RANDOX kits; UK). TS was calculated as follows: (serum iron/ TIBC) ×100 and values were expressed as percentage (%).The serum MDA level was estimated by using a reaction with thiobarbituric acid as a thiobarbituric acid reactive substance to produce a pink colored complex. Next, its fluorescence intensity was measured at 547 nm with excitation at 525 nm by a spectrofluorimeter (model SFM 25 A; Kontron, Milan, Italy).[31] Measurement of TAC in serum was performed by using the colorimetric method with commercial kits (TAC, RANDOX kits; UK).[32] All anthropometric, dietary intakes, blood sampling, and biochemical measurements were assessed again at the end of intervention period in both groups.

### *Statistical analysis*

Data were analyzed using SPSS version 16 (SPSS Inc., Chicago, IL, USA) and the results are expressed as means ± SD. Normal distribution was tested with the Kolmogorov-Smirnov test. The quantitative and qualitative variables were expressed as the mean (SD) and frequency (percentage), respectively. The baseline measurements were compared using independent samples t- test. The paired t-test was applied to compare means before and after intervention values in each group. Analysis of covariance (ANCOVA) was used to identify any differences between the two groups at the end of study, adjusting for baseline values and covariates. The percentage of changes in variables after intervention was determined with the formula: [(after values–before values)/ before values] × 100. Results with $P < 0.05$ were considered as statistically significant.

## Results

Fifty-two patients completed the 8 wk study (green tea group, n=26; control group, n=26; Fig 1).Compliance was good, more than 98% of the tea bags in a prescribed manner being consumed during the study period. Participants did not report any adverse effects or symptoms with the green tea consumption during the study.

General characteristics and dietary intakes of participants at the beginning and end of the study are reported in Table 1. There were no significant differences between or within groups in weight and BMI at the beginning of the study and after 8 wk of intervention. No significant differences in energy and other dietary intakes were observed between two groups at baseline. Total energy and nutrient intakes also did not change significantly in any of the groups during the study. The mean dose of desferrioxamine was similar in any of groups before and during the study.

Serum levels of iron, ferritin and TS value of subjects at baseline and 8 wk intervention are shown in Table 2. There were no significant differences in serum levels of iron, ferritin and TS value between two groups at baseline.

**Table 1.** General characteristics and dietary intakes of patients with β-thalassemia major at baseline and 8 wk of intervention.

| Variable | Measurement Period | Green tea group (n = 26) | Control group (n=26) |
|---|---|---|---|
| Age (y) | Baseline | 23.1 ± 3.33 | 24.2 ± 3.15 |
| Height (cm) | Baseline | 157.92 ± 5.51 | 163.15 ± 6.39 |
| Weight (kg) | Baseline | 52.08 ± 5.08 | 51.70 ± 5.47 |
| | After intervention | 51.8 ± 5.44 | 51.72 ± 5.49 |
| BMI (kg/m2) | Baseline | 20.90 ± 1.93 | 19.42 ± 1.73 |
| | After intervention | 20.78 ± 2 | 19.42 ± 1.62 |
| Desferrioxamine (mg/kg) | Throughout the study | 31.53 ± 3.39 | 33.07 ± 4.26 |
| Energy (kcal/d) | Baseline | 1838 ± 192.80 | 1884 ± 196.60 |
| | After intervention | 1713 ± 402.60 | 1776.2 ± 237.56 |
| Carbohydrate (g/d) | Baseline | 259.7 ± 47.30 | 269.6 ± 48.30 |
| | After intervention | 232.2 ± 95.50 | 251± 61.40 |
| Protein (g/d) | Baseline | 77.03 ± 26.12 | 75.58 ± 25.81 |
| | After intervention | 72.5 ± 25.07 | 72.02 ± 16.17 |
| Total fat (g/d) | Baseline | 54.59 ± 20.18 | 55.39 ± 21.07 |
| | After intervention | 54.93 ± 16.80 | 54.08 ± 12.76 |
| Iron (mg) | Baseline | 15.3 ± 3.58 | 16.7 ± 4.72 |
| | After intervention | 17.5 ± 4.86 | 15.17 ± 6.86 |
| Vitamin C (mg) | Baseline | 121.8 ± 74.16 | 106.7 ± 55.42 |
| | After intervention | 99.56 ± 46.10 | 96.19 ± 35.95 |
| Vitamin E (mg) | Baseline | 5.60 ± 10.04 | 3.34 ±2.60 |
| | After intervention | 4.26 ± 5.24 | 3.51 ± 3.26 |

BMI: body mass index, GT: green tea
Data were expressed as mean ± standard deviation

Significant differences were seen between two groups in serum levels of iron and ferritin at the end of study, adjusted for baseline values, intake of subcutaneous desferrioxamine agent and changes of weight and calorie during the study ($P < 0.05$). Serum levels of iron and ferritin significantly decreased in the intervention group by 21.15%, $P < 0.05$ (vs. 2.43%, $P = 0.72$ increase in control group) and 20.36%, $P < 0.05$ (vs. 0.9%, $P = 0.27$ decrease in control group), respectively at the end of the study. Changes in TS value were not significant within or between groups after intervention in any of groups.

Antioxidant status at baseline and after the 8wk intervention is presented in Table 3. Significant differences were seen between two groups in serum levels of MDA and TAC at baseline. There was also significant difference between two groups in serum level of MDA at the end of study adjusted for baseline MDA level, intake of subcutaneous desferrioxamine agent and changes of weight and calorie during the study. Consumption of green tea decreased serum level of MDA in green tea group by 35.26%, $P< 0.05$ (vs. 2.33%, $P=0.38$ decrease in control group). Significant difference was also detected in serum levels of TAC between two groups at the end of study adjusted for baseline TAC levels and the same covariates mentioned above. Changes in TAC level within groups were not significantly in any of studied groups throughout the study.

**Table 2.** Serum iron parameters of patients with β-thalassemia major at baseline and 8 wk of intervention.

| Variable | Measurement Period | Green tea group (n = 26) | Control group (n= 26) | MD(95%CI),P-value |
|---|---|---|---|---|
| iron(µg/dL) | Baseline | 234.7±49.3 | 298.19±66.87 | -63.46 (-96.20, -30.72), P= 0.05$^{\infty}$ |
| | After intervention | 185.9±51.6 | 300.65±53.5 | |
| | MD(95%CI), p value$^{*}$ | -48.8(-62.15,-35.45), P< 0.05$^{*}$ | 2.46(-11.29,16.22), P= 0.72 | -64.11 (-84.22, -44.00), P < 0.05 $^{\#}$ |
| ferritin(ng/dl) | Baseline | 2776.07±533.4 | 3070.23±643.6 | -294.15 (-623.45,35.14), P= 0.60$^{\infty}$ |
| | After intervention | 2187.07±496.6 | 3018.76±642.4 | |
| | MD(95%CI), p value$^{*}$ | - 589(-777.2,-400.7), P < 0.05$^{*}$ | -51.46(-144.97, 42.04), P=0.27 | -544 (-747.73, -340.26), P< 0.05$^{\#}$ |
| TS (%) | Baseline | 93.7±32.9 | 91.45 ±19.23 | 2.24 (-12.76, 17.26), P= 0.16$^{\infty}$ |
| | After intervention | 88.58 ±23.90 | 93.45 ±19.51 | |
| | MD(95%CI), p value$^{*}$ | -5.12(-14.91,4.66), P= 0.29 | 2 (-3.39,7.4), P= 0.45 | -5.29 (-14.81, 4.219), P= 0.27$^{\#}$ |

TS: transferrin saturation
$^{\infty}$MD (95% CI), P-value is reported based on the analysis of independent sample t test.
*MD (95% CI), P-value is reported based on the analysis of paired sample t test.
$^{\#}$MD (95% CI), P-value is reported based on the analysis of covariance.

**Table 3.** Antioxidant status of patients with β-thalassemia major at baseline and 8 wk of intervention.

| Variable | Measurement Period | Green tea group (n = 26) | Control group (n= 26) | MD(95%CI), P-value |
|---|---|---|---|---|
| Serum MDA(nmol/ml) | Baseline | 3.01±0.64 | 3.44±1.29 | -0.430 (-1.00, 0.13), P < 0.05$^{\infty}$ |
| | After intervention | 1.89±0.55 | 3.36±1.55 | |
| | MD(95%CI), p value$^{*}$ | -1.12(-1.43, -.815), P < 0.05$^{*}$ | -.076(-.254,0.1), P= 0.38 | -1.20 (-1.52, -0.883), P < 0.05$^{\#}$ |
| Serum TAC (nmol/l) | Baseline | 2.2665±0.77 | 2.0862 ±0.35 | 0.180 (-0.15, 0.51), P < 0.05$^{\infty}$ |
| | After intervention | 2.2619±0.48 | 1.9431 ±0.39 | |
| | MD(95%CI), p value$^{*}$ | -0.00462(-0.416, 0.407), P=0.98 | -0.14308( 0.347,0.061), P= 0.16 | 0.373 ( 0.132, 0.614), P < 0.05$^{\#}$ |

MDA: malondialdehyde ,TAC: total antioxidant capacity.
$^{\infty}$MD (95% CI), P-value is reported based on the analysis of independent sample t test.
*MD (95% CI), P-value is reported based on the analysis of paired sample t test.
$^{\#}$MD (95% CI), P-value is reported based on the analysis of covariance.

## Discussion

In *β*-thalassemia major patients with transfusion dependent anemia, iron accumulation is serious in the absence of effective iron chelation therapy.[33] Iron is a crucial cause of ROS generation in vivo and plays a key role in contributing to oxidative stress.[21]

Based on the results, the means of serum iron and ferritin levels in all of studied subjected at baseline and after intervention period were higher than normal limits (50-150 µg/dL and 10-400 ng/dl, respectively). High serum iron and ferritin levels were detected in *β*-thalassemia patients in previous studies.[16,34-38] In the study by Laksmitawatic et al. serum levels of iron and ferritin in 14 transfusion dependent *β*-thalassemia major subjects aged 11 - 25 y and 9 patients aged 17–30 y were about two-fold and 60- fold higher than of 21 healthy controls, respectively.[37] Asma Kassab-Chekir et al. reported that serum iron was increased 2-fold in 56 β-thalassemia major children aged 8±3.4 y compared to controls.[36] In another study conducted by Moayedi et.al on 119 β- thalassemia patients with mean age of about 19.4 y, serum ferritin level was in the range of 600-9400 ng/ml16. Serum ferritin concentration is directly related to body iron stores and has proved to be useful in the diagnosis of iron overload. It correlates with cardiac impairment and survival in beta thalassemia patients.[16,39]

In the blood stream, iron is transported by transferrin. In normal situation, about 30% of transferrin is saturated with iron.[40] An increased value of TS is detectable in β-thalassemia patients, due to ineffective erythropoiesis16. Based on the results, TS value of our subjects were high in both studied groups prior and after intervention. Cighetti et al. also showed that plasma TS was increased in 21 patients aged 19-39 y affected by β -thalassemia major.[41]

Our study showed that green tea consumption decreased serum iron and ferritin levels in the intervention group compared to control group. These findings are in agreement with findings of previous human and animal studies.[28-30]

TS value of our subjects did not change significantly in any of groups, throughout the study. S. Srichairatanakool et al. reported that green tea extract 2 gr % decreased TS plasma in β-thalassemia blood samples.[22] S. Ounjaijean and et al. detected that administration of green tea extract for 4 month decreased plasma TS in iron overload rats.[28]

It was demonstrated that green tea and its constituent catechins such as EGCG (with three galloyl groups) prevent iron absorption by binding to ferric iron and creating unsolvable complexes in the gastrointestinal lumen, thereby reducing the bioavailability of dietary iron.[28,42] Brune et al. examined the properties of tannin and catechin on nonheme-iron absorption. The authors determined that the inhibitory effect is more prominent when there are 3 hydroxyl groups (galloyl) on the phenolic structure.[43] Other studies also have shown that many polyphenol containing beverages such as green tea inhibit non-haem iron absorption.[42,44,45] Samman S et al. indicated that, the addition of phenolic-rich extracts of green tea and rosemary to the test meals of healthy women diminished nonheme-iron absorption by 28% and 21%, respectively.[45]

The metal binding activity of catechins; especially EGCG and ECG, also remove intrahepatic iron pools.[29] Moreover excessive plasma iron and TS in thalasemic patients can lead to the formation of non-transferrin-bound iron (NTBI).[46] The uptake of plasma NTBI into tissues can lead to excess iron accumulation and contribute to increased intracellular labile iron pools.[8,48] In vitro studies have shown that green tea extract as well as EGCG and ECG fractions can chelate plasma NTBI. Metal binding activity of phenolic compounds was also reported in other plants.[16,22,48]

Our findings confirmed the potential effects of green tea on reducing serum iron and ferritin levels in patients with β–thalassemia major. Non-significant changes in TS value might be related to higher baseline values of serum iron in studied subjects.

Oxidative stress is the outcome of an inequity between free radical production and decreased degradation.[49] An elevated oxidant stress and a reduced antioxidant status stimulate peroxidative damage to cell and other organelle membranes. It is well recognized that defects of oxidant-antioxidant balance occur in *β*-thalassemia.[50] Evidences show that MDA, a product of lipid peroxidation is generated in extra amounts in *β*-thalassemia patients.[35,41,51-55] Elevated MDA may be related to existing large amount of membrane bound iron in *β*-thalassemia erythrocytes, which is promoted by iron overload through frequent blood transfusions and consequent oxidative stress produced by ROS.[4,8,56] Iron is involved in many oxidative stress related pathways and is the prime generator of H2O2 and •OH that damages DNA and other biomolecules.[57-59] Lower TAC levels were detected in *β*-thalassemia patients.[34,60,61] TAC accounts as an index of the general protective effect of antioxidants in body fluids and other compounds of cells against oxidative injury.[62] Lowered level of TAC might be result of its utilization for counteracting the action of ROS and iron overload.[35]

According to our findings, green tea consumption caused a considerable decrease in serum MDA levels. It also maintained TAC levels in treated group. Our results were comparable with findings

of some previous studies.[22,28,30] S. Srichairatanakool et al. indicated that green tea extract (2 gr %) decreased MDA levels in β-thalassemia blood samples.[22] In the study by Amir Nili Ahmadabadi et al. serum lipid peroxidation decreased in β-thalassemia major patients after one month consumption of green tea.[30] Ounjaijean et al. showed that administration of green tea extract decreased serum MDA in iron-loaded rats.[28] No published data are available about effects of green tea on TAC levels in patients with β-thalassemia major.

Antioxidant properties of polyphenol compounds were determined in other studies.[63] Kawabata et al. and Yoshino et al. demonstrated that several polyphenols have capability to chelate iron and protect rat microsomes from lipid peroxidation by blocking the Fenton reaction.[64,65] Anghileri et al. have shown that polyphenols from green tea protect against lipid peroxidation of mouse liver tissue suspensions.[66] Iron-binding by polyphenol compounds results in cytoprotective effects, and that both iron binding and lipophilicity are noticeable factors contributing to antioxidant activity.[21]

Our findings indicated the positive effects of green tea in reducing oxidative stress in studied patients. Such valuable results might be due to iron-chelating capacity of green tea and subsequent lowered serum iron and ferritin levels which were demonstrated in the treated group.

It should be note that, there were no significant changes in BMI, dietary intakes and adherence of desferrioxamine among subjects during the study .Thus, these factors could not be considered as confounding factors in the interpretation of our results. It was also anticipated that the randomized design could balance probable bias between the study and control groups. Some of our study limitations were its single blind design and short study duration of 8 wk. So, the interpretation of the results of this study may not be applicable for using other amounts of green tea, other intervention periods or for such patients with different age ranges. Considering that NTBI as pool of free iron is potentially dangerous through generating ROS and may lead to serious tissue injury,[67] so other studies are warranted to investigate the effects of green tea on this parameter in β-thalassemia major patients, too.

## Conclusion

As a conclusion, short term intake of green tea had valuable effects on parameters of serum iron and antioxidant status in β-thalassemia major patients. Further studies with a longer intervention period are needed to show considerable clinical improvements.

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## Conflict of interests

The authors claim that there is no conflict of interest.

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