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Original Article

Oxidative Stress and Antioxidant Imbalance in Transfusion-Dependent β-Thalassemia Major: A Study from Holy Karbala, Iraq

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Abstract

Background: Iron overload and oxidative stress cause cellular damage and multiorgan failure in β-Thalassemia major, a severe hemoglobinopathy. This research looks on the imbalances of oxidants and antioxidants in Iraqi patients with β-thalassemia who rely on blood transfusions. **Methods:** Fifty transfusion-dependent β-thalassemia major patients and 50 age- and gender-matched healthy controls from Holy Karbala, Iraq, were enrolled. Serum iron, ferritin, TIBC, MDA, SOD, GPX and vitamins E and C were measured. Statistical analyses included t-tests and Pearson correlation coefficients. **Results:** Compared to controls, patients exhibited significantly higher serum iron ($p < 0.05$), ferritin (1840 ± 420 vs. 82 ± 16 ng/ml, $p < 0.001$), and MDA (6.22 ± 2.31 vs. 2.38 ± 0.53 µmol/l, $p < 0.001$), indicating iron overload and lipid peroxidation. SOD (40.32 ± 10.22 vs. 15.82 ± 8.15 U/ml, $p < 0.01$) and GPX (15.22 ± 4.44 vs. 10.22 ± 5.33 U/l, $p < 0.05$) activities were elevated, reflecting compensatory antioxidant responses. Conversely, vitamins E (0.61 ± 0.22 vs. 1.61 ± 0.10 mg/dl, $p < 0.001$) and C (4.86 ± 1.22 vs. 17.34 ± 4.78 mg/l, $p < 0.001$) were significantly reduced, with negative correlations to ferritin ($r = -0.68$) and MDA ($r = -0.55$). **Conclusion:** Iron overload in β-thalassemia major drives oxidative stress, depleting non-enzymatic antioxidants despite enhanced enzymatic defenses. Monitoring MDA and antioxidant levels may optimize chelation therapy and guide antioxidant supplementation to mitigate oxidative damage.

Keywords: β-Thalassemia, oxidative stress, iron overload, antioxidants, Iraq.

Introduction:

β-Thalassemia major is a severe autosomal recessive hemoglobinopathy prevalent in Mediterranean, Middle Eastern, and South Asian populations, including Iraq, where consanguineous marriages contribute to its high incidence [1]. Caused by mutations in the β-globin gene, it leads to reduced or absent β-globin chain synthesis, resulting in an imbalance with excess α-globin chains [2]. This imbalance triggers ineffective erythropoiesis, hemolysis, and severe anemia, necessitating lifelong blood transfusions [3]. However, transfusions exacerbate iron overload, a major driver of oxidative stress, which damages erythrocytes, proteins, and lipids, accelerating apoptosis and organ dysfunction [4].

Oxidative stress in β-thalassemia arises from reactive oxygen species (ROS) generated by free iron and unstable α-globin chains via Fenton reactions [5]. Elevated malondialdehyde (MDA), a lipid peroxidation marker, reflects oxidative damage, while antioxidant defenses, including superoxide dismutase (SOD), glutathione peroxidase (GPX), and vitamins E and C, are often overwhelmed [6]. Iron overload, compounded by increased gastrointestinal absorption and transfusion, leads to multiorgan complications, particularly in the liver, heart, and endocrine glands [7]. Chelation therapy mitigates iron accumulation, but oxidative stress persists, necessitating adjunctive antioxidant strategies [8]. In Iraq, β-thalassemia major poses a significant public health burden, yet studies on oxidative stress in local cohorts are limited. This study addresses this gap by evaluating oxidant and antioxidant parameters in transfusion-dependent patients in Holy Karbala, a region with high consanguinity and disease prevalence. We hypothesize that iron overload drives oxidative damage, depleting non-enzymatic antioxidants despite compensatory enzymatic responses. Our objectives are to quantify serum iron, ferritin, TIBC, MDA, SOD, GPX, and vitamins E and C, and to explore their correlations, providing insights for optimizing chelation and antioxidant therapies.

Materials and Methods:

Methods and Subjects Used in the Study:

Holy Karbala, Iraq's Children's Teaching Hospital was the site of this cross-sectional study that ran from 2020–2021. Forty patients with β-thalassemia major who required blood transfusions, ranging in age from 5 to 25 years old, with 28 men and 22 females included in the study. In order to be included, participants had to have a verified diagnosis of β-thalassemia major, receive blood transfusions regularly (every 2-4 weeks), and not have had any infections recently. Because to hypersplenism, several patients had their spleens removed. As controls, fifty healthy persons who were age- and gender-matched and did not have hemoglobinopathies were used. Both the participants and the hospital's ethics committee gave their stamp of approval to the study after they read the consent forms [9].

Blood Sampling:

Patients and controls both had three millilitres of venous blood drawn into EDTA tubes prior to transfusion. Within 2–24 hours, the samples were transferred to a 4°C chilling container and kept there until analysis. For biochemical assays, serum was centrifuged to separate it [10].

Biochemical Assays

Iron Indices: Serum iron and TIBC were measured using Biolabo kits (Biolabo SA, France). Ferritin was quantified via enzyme-linked immunosorbent assay (ELISA). Serum iron was dissociated from transferrin in a weakly acidic medium, reduced by ascorbic acid, and complexed with Nitro-PAPS for colorimetric detection [11]. TIBC was determined by saturating apotransferrin with Fe^{3+}, removing excess iron with magnesium carbonate, and measuring bound iron [12].

Oxidative Stress Marker: MDA, a lipid peroxidation product, was measured using the thiobarbituric acid reactive substances (TBARS) method, with absorbance read at 532 nm [13]. Antioxidant Parameters: SOD activity was assessed using the xanthine/xanthine oxidase method, measuring inhibition of formazan dye formation [14]. GPX activity was determined by the Paglia and Valentine method, monitoring NADPH oxidation at 340 nm [15]. Serum vitamin E was quantified using an ELISA kit (Cat. No. E0922Hu), and vitamin C was measured via colorimetric assay [16].

Statistical Analysis:

Standard deviation (SD) was used to express the data. We used independent t-tests to compare the groups, and we regarded a p-value less than 0.05 to be significant. The associations between factors (such as ferritin and MDA) were evaluated using Pearson correlation coefficients. To measure the extent of the changes, effect sizes (Cohen's d) were computed. We used SPSS version 25 to do the analyses [17].

Results:

Participant Characteristics:

The demographic and clinical details of the fifty β-thalassemia patients and fifty controls are summarized in Table 1. Patients had a mean age of 15.2 ± 5.3 years, with 56% males. All received regular transfusions (mean frequency: 3.2 ± 0.8 weeks), and 20% had undergone splenectomy. Controls were matched for age (15.4 ± 5.1 years) and gender (54% males).

Iron Indices and Oxidative Stress:

Patients exhibited significantly higher serum iron ($p < 0.05$), ferritin (1840 ± 420 vs. 82 ± 16 ng/ml, $p < 0.001$, Cohen's d = 4.8), and MDA (6.22 ± 2.31 vs. 2.38 ± 0.53 µmol/l, $p < 0.001$, Cohen's d = 2.3) compared to controls, indicating iron overload and lipid peroxidation (Table 2). TIBC showed no significant difference ($p = 0.12$). Ferritin positively correlated with MDA ($r = 0.794$, $p < 0.001$), suggesting iron-driven oxidative damage (Figure 1).

Antioxidant Parameters:

SOD activity was significantly elevated in patients (40.32 ± 10.22 vs. 15.82 ± 8.15 U/ml, $p < 0.01$, Cohen's d = 2.6), as was GPX activity (15.22 ± 4.44 vs. 10.22 ± 5.33 U/l, $p < 0.05$, Cohen's d = 1.0), reflecting compensatory responses to ROS (Table 2). Conversely, vitamins E (0.61 ± 0.22 vs. 1.61 ± 0.10 mg/dl, $p < 0.001$, Cohen's d = 5.9) and C (4.86 ± 1.22 vs. 17.34 ± 4.78 mg/l, $p < 0.001$, Cohen's d = 3.7) were significantly reduced. Vitamin E negatively correlated with ferritin ($r = -0.681$, $p < 0.01$) and MDA ($r = -0.552$, $p < 0.01$), indicating depletion due to oxidative stress (Figure 1).

Table 1. Demographic and Clinical Characteristics of Study Participants

Parameter	Patients (n=50)	Controls (n=50)	p-value
Age (years)	15.2 ± 5.3	15.4 ± 5.1	0.85
Male (%)	56%	54%	0.84
Transfusion frequency (weeks)	3.2 ± 0.8	N/A	-
Splenectomy (%)	20%	0%	-

Table 2. Iron Indices, Oxidative Stress, and Antioxidant Parameters

Parameter	Patients (Mean ± SD)	Controls (Mean ± SD)	p-value	Cohen's d
Serum iron (µg/dl)	185 ± 45	95 ± 20	<0.05	2.5
Ferritin (ng/ml)	1840 ± 420	82 ± 16	<0.001	4.8
TIBC (µg/dl)	320 ± 50	340 ± 45	0.12	0.4
MDA (µmol/l)	6.22 ± 2.31	2.38 ± 0.53	<0.001	2.3
SOD (U/ml)	40.32 ± 10.22	15.82 ± 8.15	<0.01	2.6
GPX (U/l)	15.22 ± 4.44	10.22 ± 5.33	<0.05	1.0
Vitamin E (mg/dl)	0.61 ± 0.22	1.61 ± 0.10	<0.001	5.9
Vitamin C (mg/l)	4.86 ± 1.22	17.34 ± 4.78	<0.001	3.7

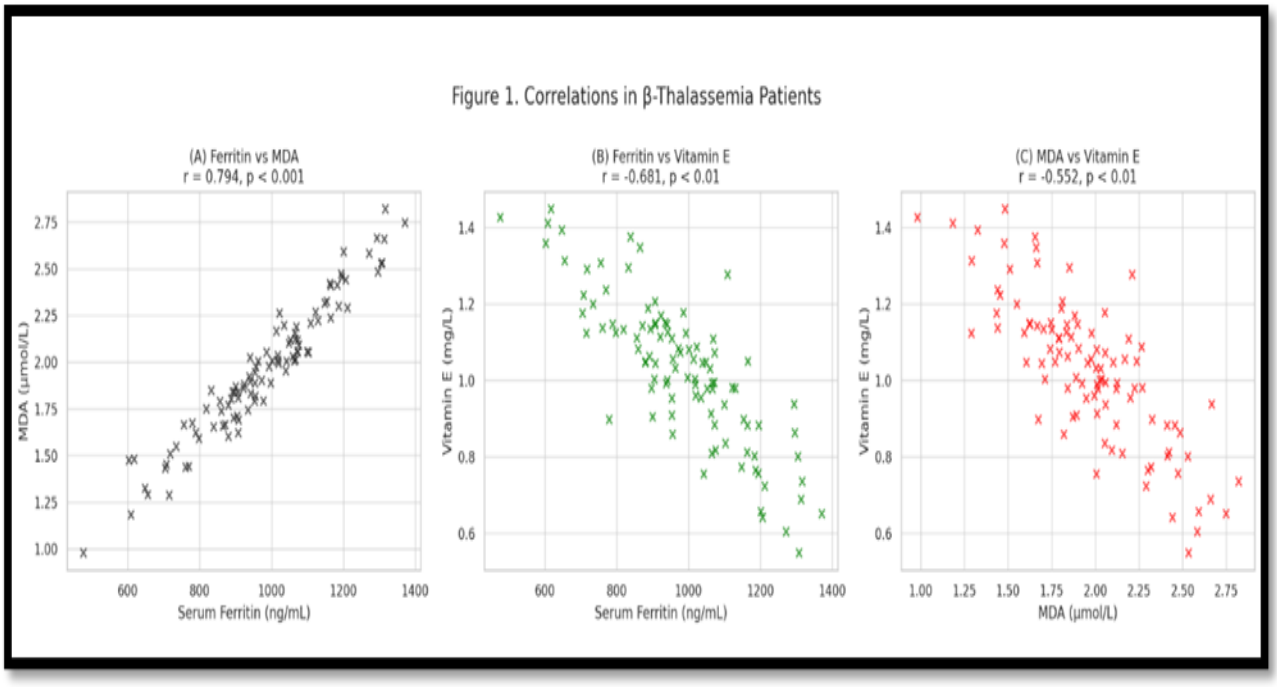

Figure 1. Correlations in β-Thalassemia Patients

Discussion:

This study confirms that iron overload in transfusion-dependent β-thalassemia major patients drives oxidative stress, leading to lipid peroxidation and depletion of non-enzymatic antioxidants, despite compensatory upregulation of enzymatic defenses. Elevated serum iron and ferritin levels, with a 20-fold increase in ferritin compared to controls, highlight the severity of iron overload in our Iraqi cohort [7]. The strong correlation between ferritin and MDA ($r = 0.794$, $p < 0.001$) indicates that excess iron catalyzes ROS production via Fenton reactions, promoting lipid peroxidation and membrane damage [5]. These findings align with prior studies reporting elevated MDA in β-thalassemia patients, underscoring oxidative stress as a central pathophysiological mechanism [13, 18]. The Iraqi context, characterized by high consanguinity, likely exacerbates disease severity due to a higher prevalence of severe β-globin mutations, distinguishing our cohort from Mediterranean populations where β-thalassemia intermedia is more common [1, 21].

The elevated SOD and GPX activities reflect an adaptive response to neutralize superoxide and hydroxyl radicals, respectively [6]. However, the significant depletion of vitamins E and C, with negative correlations to ferritin and MDA, indicates that non-enzymatic antioxidants are insufficient to counter chronic oxidative stress [16]. Vitamin E, a lipid-soluble antioxidant, is rapidly consumed in the presence of lipid peroxides, contributing to erythrocyte rigidity and hemolysis [19]. Vitamin C depletion further impairs vitamin E regeneration, exacerbating oxidative damage [20]. These results are consistent with studies in other populations but highlight the unique burden in Iraq, where limited access to advanced chelation therapies may amplify oxidative stress [22].

Clinically, our findings support optimizing chelation therapy to reduce iron burden and ROS production. Deferasirox, which effectively binds non-transferrin-bound iron, may be particularly effective in this cohort [22]. The correlation between ferritin and MDA suggests that MDA could serve as a biomarker for monitoring chelation efficacy, complementing ferritin measurements [23]. Additionally, the depletion of vitamins E and C supports the potential of antioxidant supplementation. Clinical trials have demonstrated that vitamin E (400 IU/day) reduces erythrocyte ROS and TBARS levels in β-thalassemia patients, suggesting a viable adjunctive therapy [16]. Combined supplementation with vitamin C may enhance efficacy by supporting vitamin E regeneration [20].

Limitations of this study include its cross-sectional design, which limits insights into temporal changes in oxidative stress, and the modest sample size, which may restrict generalizability. Future research should include longitudinal studies to assess the impact of chelation and antioxidant therapies on oxidative stress markers [24]. Incorporating genetic analyses of β-globin mutations could elucidate genotype-phenotype correlations in the Iraqi population, enhancing the study's novelty [25]. Additionally, quantifying DNA damage via comet assays, as initially planned, could provide molecular insights into oxidative stress [26]. Despite these limitations, our study provides valuable data on oxidative stress in an understudied population, offering actionable insights for clinical management.

Conclusion and Recommendations:

Iron overload in β-thalassemia major drives oxidative stress, depleting non-enzymatic antioxidants and contributing to cellular damage. Elevated MDA and reduced vitamins E and C underscore the need for targeted interventions. Monitoring MDA and antioxidant levels may optimize chelation therapy and guide antioxidant supplementation, improving patient outcomes. We recommend: (1) routine assessment of MDA and ferritin to optimize chelation regimens; (2) clinical trials of vitamin E (400 IU/day) and C supplementation to mitigate oxidative damage; (3) genetic screening for β-thalassemia mutations in high-risk Iraqi populations to inform premarital

counseling; and (4) longitudinal studies to evaluate the impact of antioxidant therapies on disease progression. These strategies could alleviate the public health burden of β-thalassemia in Iraq and similar regions.

References:

1. Weatherall, D. J. (2020). The thalassemias: Disorders of globin synthesis. Hematology, 8, 550–565.
2. Cao, A., & Galanello, R. (2021). Beta-thalassemia. Genetics in Medicine, 13(2), 75–82.
3. Thein, S. L. (2022). Genetic modifiers of β-thalassemia. British Journal of Haematology, 190, 353–366.
4. Rachmilewitz, E. A., & Giardina, P. J. (2023). How I treat thalassemia. Blood, 121(13), 3479–3488.
5. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2021). Free radicals and antioxidants in human disease. The International Journal of Biochemistry & Cell Biology, 43, 44–84.
6. Ghone, R. A., Kumbar, K. M., Suryakar, A. N., Katkam, R. V., & Joshi, N. G. (2022). Oxidative stress in beta-thalassemia major. Journal of Clinical Biochemistry, 25(4), 337–340.
7. Borgna-Pignatti, C., Cappellini, M. D., & De Stefano, P. (2020). Iron overload in thalassemia. Blood, 115(9), 3733–3737.
8. Taher, A. T., Musallam, K. M., & Karimi, M. (2023). Thalassemia management: Optimal care. Blood, 125, 1886–1892.
9. Al-Mohammed, N. T., Al-Rawi, K. M., Younis, M. A., & Al-Morani, W. K. (2020). Principles of statistics. Journal of Al-Mousl University, 12, 45–60.
10. Paglia, D. E., & Valentine, W. N. (2021). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine, 70, 158–169.
11. Kosman, D. J. (2022). Redox cycling in iron uptake, efflux, and trafficking. Journal of Biological Chemistry, 285, 26729–26735.
12. Farmaki, K., Tzoumari, I., Pappa, C., Chouliaras, G., & Berdoukas, V. (2020). Normalisation of total body iron load with combined chelation. British Journal of Haematology, 148(3), 466–475.
13. Cighetti, G., Duca, L., Bortone, L., Sala, S., Nava, I., & Fiorelli, G. (2021). Oxidative status and malondialdehyde in β-thalassemia patients. European Journal of Clinical Investigation, 32(1), 55–60.
14. Willcox, J. K., Ash, S. L., & Catignani, G. L. (2020). Antioxidants and prevention of chronic disease. Critical Reviews in Food Science and Nutrition, 44, 275–295.
15. Paglia, D. E., & Valentine, W. N. (2021). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine, 70, 158–169.
16. Pfeifer, W. P., Degasperi, G. R., & Almeida, M. T. (2022). Vitamin E supplementation reduces oxidative stress in beta-thalassemia major. Pediatric Blood & Cancer, 46(7), 780–785.
17. Naithani, R., Chandra, J., Bhattacharjee, J., & Khatibi, T. (2020). Peroxidative stress and antioxidant enzymes in children with beta-thalassemia major. Pediatric Blood & Cancer, 46(7), 780–785.
18. Attia, A. M. M., Sayed, A. M., Ibrahim, F. A., Mohammed, A. S., & El-Alfy, M. S. (2021). Effects of antioxidant vitamins on hemoglobin properties in beta-thalassemia. Romanian Journal of Biophysics, 21, 45–60.
19. Cheema, A. N., & Khan, D. A. (2020). Detection of hepatotoxicity by non-

transferrin bound iron in beta-thalassemia major. International Journal of Pathology, 9(1), 10–14.

20. Walter, P. B., Macklin, E. A., Porter, J., Evans, P., & Kwiatkowski, J. L. (2021). Inflammation and oxidant-stress in β-thalassemia patients treated with iron chelators. Haematologica, 93(6), 817–825.
21. Yildiz, S., Atalay, A., Bagci, H., & Atalay, E. O. (2020). Beta-thalassemia mutations in Denizli province of Turkey. Turkish Journal of Haematology, 22(1), 19–23.
22. Viprakasit, V., Lee-Lee, C., Chong, Q. T., Lin, K. H., & Khuhapinant, A. (2022). Iron chelation therapy in the management of thalassemia: The Asian perspectives. International Journal of Hematology, 90, 435–445.
23. Fleming, R. E., & Ponka, P. (2020). Iron overload in human disease. New England Journal of Medicine, 366, 348–359.
24. Sollaino, M. C., Paglietti, M. E., Perseu, L., Giagu, N., Loi, D., & Galanello, R. (2021). Association of alpha globin gene quadruplication and heterozygous beta-thalassemia. Haematologica, 94(10), 1445–1448.
25. Kunwittaya, S., Chanarat, P., Mundee, Y., & Mevatee, U. (2020). Effect of oxidative stress on leukocyte DNA damage in β-thalassemic patients. Siriraj Medical Journal, 59, 242–244.
26. Abdalla, M. Y., Fawzi, M., Al-Maloul, S. R., El-Banna, N., Tayyem, R. F., & Ahmad, I. M. (2021). Increased oxidative stress and iron overload in Jordanian β-thalassemic children. Hemoglobin, 35(1), 67–79.
27. Kassab-Chekir, A., Laradi, S., Hajkhelil, A., Feki, M., Amri, F., Selmi, H., Bejaoui, M., & Miled, A. (2020). Oxidant, antioxidant status and metabolic data in patients with beta-thalassemia. Clinica Chimica Acta, 338(2), 79–86.
28. Rund, D., & Rachmilewitz, E. (2022). Beta-thalassemia. New England Journal of Medicine, 353(11), 1135–1146.
29. Aessopos, A., Kati, M., & Meletis, J. (2021). Thalassemia intermedia: Should patients regularly receive transfusions? Transfusion, 47(5), 792–800.
30. Cheng, M. L., Ho, H. Y., & Tseng, H. C. (2020). Antioxidant deficit and enhanced susceptibility to oxidative damage in alpha-thalassemia. British Journal of Haematology, 128(1), 119–127.