Effects of a Three-Month-Antioxidant Therapy on the Oxidative Balance in Beta-Thalassemia Patients

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Abstract

Background and aim: Beta-thalassemia is one of the most common single-gene disorders worldwide. Depending on the amount of genetic defect, patients may present with mild to severe clinical phenotype. Precipitation of excess α-chains induces the production of free radicals and reactive oxygen species. Antioxidants play an important role in reducing oxidative damage. This prospective, interventional case-control clinical trial investigated the impact of a three-month antioxidant therapy regimen, consisting of vitamin E and N-acetylcysteine (NAC), on oxidative balance in patients with transfusion-dependent Beta-thalassemia (TDT). The primary aim was to evaluate changes in oxidative stress biomarkers—total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI)—alongside secondary outcomes, such as hematological parameters, serum ferritin and organ-function parameters.

Patients and Methods: The study was conducted at Al-Hussein University Hospital and El-Obour Hospital, Kafr El-Sheikh Governorate, during the period from November 2022 to November 2023, the study included 88 TDT patients, aged 5–18 years, divided by systematic random method into a case group (n=48) receiving antioxidants alongside standard treatment and a control group (n=40) receiving only standard treatment. Eligible patients underwent a comprehensive baseline assessment which included medical history, physical examination and laboratory investigations (CBC, serum ferritin, organ-function tests, and oxidative stress biomarkers). At the end of the three-month period, a repeat comprehensive follow up assessment was conducted. Post-treatment, the case group exhibited a highly significant increase in TAC, decrease in TOS and reduction in OSI. Significant improvements were noted in serum ferritin and organ-function parameters reflecting reduced oxidative damage to these organs.

Conclusion: A three-month regimen of vitamin E and NAC significantly improved oxidative damage in TDT patients, as evidenced by increased TAC, reduced TOS and OSI, lower serum ferritin, and better renal and liver functions. While it does not directly ameliorate anemia, the therapy offers a protective effect against oxidative damage, supporting its potential inclusion in TDT patients' management protocols.

Keywords: Antioxidant; Beta-Thalassemia; Oxidative Stress.

Introduction

Beta-thalassemia is one of the most common single-gene disorders worldwide. It is characterized by the absence or decrease in the β-globin chain synthesis due to a mutation in the β-globin gene. Depending on the amount of genetic defect, patients may present with mild to severe clinical phenotype. In severe form, transfusion-dependent Beta-thalassemia (TDT), patients need regular blood transfusions since early infancy due to severe hemolytic anemia and ineffective erythropoiesis[(1)]. Precipitation of excess α-chains also induces the production of free radicals and reactive oxygen species, which in turn weakens the antioxidant defense system and leads to hemolysis due to oxidation of different red blood cell components[(2)]. TDT patients face iron overload due to lifelong blood transfusions on one hand as well as hemolysis and ineffective erythropoiesis on the other hand. Precipitation of excess iron in the heart, liver, and endocrine systems causes higher mortality and morbidity which necessitates using iron chelation therapy in these patients. Iron is recognized to be a catalyst in developing reactive oxygen species. Oxidative stress is a major mechanism contributing to the progression of the disease in TDT[(3)]. Antioxidants play an important role in reducing the oxidative damage. Antioxidants are endogenous and exogenous agents consisting of enzymes such as catalase, superoxide dismutase, and glutathione peroxidase and reductase; large molecules including ferritin and albumin; and small molecules such as uric acid, bilirubin, ascorbic acid, reduced glutathione, α-tocopherol, and vitamin E[(4)]. The oxidant and antioxidant status in humans can be assessed either by measuring separate agents in cells and plasma or by global assessment of the total oxidant status (TOS) and total antioxidant capacity (TAC)[(5-6)].

Study procedures

This prospective, interventional case-control clinical trial investigated the impact of a three-month antioxidant therapy regimen, consisting of vitamin E and N-acetylcysteine (NAC) on oxidative balance in TDT patients. The primary aim was to evaluate changes in oxidative stress biomarkers—TAC, TOS, and OSI—alongside secondary outcomes, such as hematological parameters, serum ferritin and organ-function parameters. It included a total of 88 TDT patients attending the Pediatric Hematology Units of Al-Hussein University Hospital, Faculty of Medicine, Al-Azhar University, Cairo, and El-Obour Hospital, Kafr El-Sheikh Governorate, the study period spanned from November 2022 to November 2023, divided by systematic random method into two groups, a case group (n=48) patients, received a three-month course of antioxidant therapy alongside the standard hospital treatment, and a control group (n=40 patients, received only the standard hospital treatment.

Ethical Considerations: The study was approved by the Ethical Committee of Al-Azhar Faculty of Medicine.

- A written informed consent was obtained from all patients and/or their parents before getting them involved in the study.
- The aims, the steps of the study, the potential benefits and hazards, all were discussed with the patients.
- Confidentiality of all data was ensured.
- The patients had the right to withdraw from the study at any time without giving reasons.
- The authors declared no fund regarding study or publication.
- The authors declared no conflict of interest regarding the study or publication.

Sample Size: The sample size was calculated based on the assumption that antioxidant therapy would have a significant impact on oxidative balance in β-thalassemia major patients. Using a 95% confidence level with a Z-score of 1.96 and a margin of error set at 5%, a minimum of 88 patients was determined to be sufficient to detect statistically significant differences between case and control groups.

Sample size was calculated using the following formula:

$$n_0 = \frac{Z^2 \cdot p \cdot (1-p)}{E^2}$$

Where:

- n_0 = sample size for infinite population.
- Z = Z score.
- P = population proportion (Assumed as 50% or 0.5).
- E = Margin of error.

Note: Z score is determined based on the confidence level.

Confidence Level: Probability that the value of a parameter falls within a specified range of values. For example, for 95% confidence level Z score is 1.960. The margin of error: It is defined as a small amount that is allowed for in case of miscalculation or change of circumstances. Generally, the margin of error is taken as 5% or 0.05.

Inclusion Criteria:

- Patients diagnosed with TDT.
- Aged between 5 and 18 years.
- Regularly receiving blood transfusions and undergoing iron chelation therapy.

Exclusion Criteria:

- Age below 5 years or above 18 years.
- History of renal or liver disease.
- Positive virology screen.
- Recent change in blood transfusion frequency or pattern within the three months prior to the study.
- Recent changes in the type or dosage of iron chelation therapy within the three months

prior to the study.

Eligible patients underwent a comprehensive baseline assessment which included:

1. Detailed patient history with strength on frequency of blood transfusion and chelation therapy.
2. Thorough physical examination (general and local) with strength on anthropometric measurements, vital signs, and organ-system examination (cardiovascular, pulmonary, gastrointestinal, hepatobiliary, hematopoietic, nephrology & urology, skin and musculoskeletal).
3. Laboratory investigations (CBC, serum ferritin, liver, renal, and thyroid function tests, random blood glucose, virology screen, and oxidative balance measurements). The total antioxidant capacity (TAC) was measured using the colorimetric method, by ERMA INC, Spectrophotometer, Japan, with assay kits (Bio diagnostic®, Egypt)[7]. The total oxidant status (TOS) was measured using the colorimetric method, by ERMA INC, Spectrophotometer, Japan, with assay kits (Elabscience®, Texas, USA)[8]. The oxidative stress index (OSI) was calculated using the following formula:

OSI = {(TOS, μmol H_2O_2 Equiv./L)/(TAC, μmol Trolox Equiv./L)}[9].

Methods: Patients in the case group received a three-month antioxidant therapy regimen alongside the standard hospital treatment, which included regular blood transfusions and iron chelation therapy.

The antioxidant therapy consisted of:

1. Vitamin E: Administered orally at a dose of 10 mg/kg/ day, up to a maximum dose of 400 mg daily.

A small amount of yoghurt was diluted with a small amount of water, then 20 mL of the previously prepared emulsion was mixed with one capsule containing 400 mg of vitamin E to obtain a final concentration of 20 mg/mL.

2. N-acetylcysteine (NAC): Administered orally at a dose of 10 mg/ kg/day, up to a maximum dose of 600 mg daily.

N-acetylcysteine 600 mg sachet was dissolved in 60 mL of water to obtain a final concentration of 10 mg/mL.

Throughout the study, patients in both the case and control groups continued to receive regular blood transfusions and iron chelation therapy as per the standard care protocol. Baseline assessments were conducted before the intervention began, and follow-up assessments were conducted at the end of the three-month period to evaluate changes in the oxidative balance biomarkers.

Post-intervention Assessment: At the end of the three-month period, a repeat comprehensive follow-up assessment was conducted, mirroring the baseline assessment tests.

Statistical Analysis: Data were analysed by the Statistical Package for the Social Sciences (SPSS) software, version 23 (SPSS Inc., Chicago, IL, USA). Descriptive data were presented as mean, standard deviation, frequency, and percentage, comparison of quantitative variables was done by Student's t-test between two groups and Analysis of Variance (ANOVA) among more than two groups, the qualitative variables were compared by the chi-square test among different groups, Pearson's correlation coefficient was calculated to assess the correlation between two quantitative variables, and multiple linear

regression was performed to determine independent variables influencing TOS and TAC.
Probability of Results: P>0.05 was considered non-significant, P<0.05 was considered significant, and P<0.001 was considered highly significant [10].

Results

Our results will be demonstrated in the following tables and figures.

Table (1): Demographic and Clinical Data of the Study Groups

Variable	Parameter	Case group n=48	Control group n=40	p-value
Age (years)	Mean ± SD	9.60 ± 3.723	9.43 ± 4.169	0.832
	Min-max	5 - 16	5 - 18	
Sex [n (%)]	Male	33 (68.7%)	21 (52.5%)	0.119
	Female	15 (31.3%)	19 (47.5%)	
Weight (kg)	Mean ± SD	30.71 ± 8.85	29.88 ± 9.025	0.664
	Min-max	20 - 50	21 – 53	
Mean weight (Z score)	Mean ± SD	0.042 ± 0.99	-0.051 ± 1.01	0.664
Height (cm)	Mean ± SD	129.98 ± 15.402	128.95 ± 15.76	0.758
	Min-max	107 - 150	112 – 167	
Mean height (Z score)	Mean ± SD	0.03 ± 0.99	-0.036 ± 1.01	0.758
Liver span (cm)	Mean ± SD	10.33 ± 1.73	11.84 ± 1.78	0.32
	Min-max	10 - 14	10 – 13	
Splenomegaly (cm)	Mean ± SD	11.95 ± 1.39	12.1 ± 1.2	0.66
	Min-max	11 - 16	11 – 16	
Splenectomy [n (%)]	Yes	4 (8.3%)	4 (10%)	0.484

*P significant if <0.05, **P highly significant if <0.001.

There was no statistical significant differences between the study groups as regards age, sex, weight, height, liver span, splenomegaly, and splenectomy (P>0.05), denoting that both groups were properly cross matched (Table 1)

Table (2): Hematological Parameters among the Study Groups, at Baseline

Variable	Parameter	Case group n=48	Control group n=40	Normal range (Doms, 2024) [11]	p-value
Hb (g/dL)	Mean ± SD	6.86 ± 1.103	6.92 ± 1.008	11.5 - 15.5	0.803

	Min-max	5.10 - 8.70	5.10 - 8.70		
HCT (%)	Mean ± SD	22.74 ± 3.15	21.95 ± 3.197	35 - 45	0.245
	Min-max	15.9 - 25.9	15.9 - 25.9		
RBCs ($\times 10^6/\mu L$)	Mean ± SD	2.99 ± 0.54	3.00 ± 0.58	4.0 - 5.2	0.991
	Min-max	2.17 - 4.01	2.1 - 4.01		
MCV (fL)	Mean ± SD	69.6 ± 6.37	67.85 ± 6.64	77 - 95	0.314
	Min-max	58.4 - 80	58.4 - 86.3		
MCH (pg)	Mean ± SD	23.135 ± 3.78	23.39 ± 3.5	25 – 33	0.739
	Min-max	17.6 - 28.4	17.1 - 30.5		
MCHC (g/dL)	Mean ± SD	31.63 ± 1.15	31.81 ± 0.76	31 - 37	0.389
	Min-max	30 - 33.4	30 - 32.9		
PLT ($\times 10^9$/L)	Mean ± SD	418.29 ± 137.7	414.35 ± 102.54	150 – 450	0.24
	Min-max	180 - 602	178 - 630		
WBCs ($\times 10^9$/L)	Mean ± SD	10.21 ± 5.25	9.55 ± 2.94	4.3 - 11.0	0.16
	Min-max	3.2 - 21.7	3.2 - 15		
Ferritin (ng/mL)	Mean ± SD	1226.96 ± 453.38	1272.73 ± 349.55	13.7 - 78.8	0.354
	Min-max	760 - 1981	760 - 1280		

*P significant if <0.05, **P highly significant if <0.001.

The Hb, HCT, RBCs, MCV, and MCH values among the case and control groups were significantly lower than the normal ranges, while MCHC, PLT and WBC were within the normal ranges, but serum ferritin values were higher than the normal ranges (Table 2)

Table (3): Organ-Function Parameters among the Study Groups, at Baseline

Variable	Parameter	Case group n=48	Control group n=40	Normal range (Doms, 2024)[11]	p-value
Urea (mg/dL)	Mean ± SD	24.81 ± 6.18	27.75 ± 11.3	18 - 45	0.126
	Min-max	18 - 37	18 - 57		
Creatinine (mg/dL)	Mean ± SD	0.688 ± 0.147	0.682 ± 0.102	0.3 - 0.7	0.841
	Min-max	0.49 - 0.92	0.55 - 0.8		
ACR (mg/g)	Mean ± SD	12.35 ± 6.43	14.02 ± 3.66	Up to 30	0.149
	Min-max	0.94 - 22.6	6.36 - 22.6		
ALT (U/L)	Mean ± SD	59.84 ± 17.23	57.23 ± 19.8	10 – 35	0.543
	Min-max	30 - 89	29 - 90		
AST (U/L)	Mean ± SD	31.92 ± 9.56	31.23 ± 11.76	15 – 40	0.762
	Min-max	19 - 52	19 - 52		
Total bilirubin	Mean ± SD	3.27 ± 2.62	3.14 ± 1.96	0.6 - 1.4	0.547

(mg/dL)	Min-max	2.26 - 3.96	2.15 - 3.84		
Direct bilirubin (mg/dL)	Mean ± SD	0.2 ± 0.04	0.19 ± 0.09	0.1 - 0.3	0.156
	Min-max	0.1 - 0.3	0.1 - 0.3		
Random blood glucose (mg/dL)	Mean ± SD	155.24 ± 16.95	162.24 ± 10.59	≤200	0.364
	Min-max	70 - 177	66 - 177		
TSH (mU/L)	Mean ± SD	3.26 ± 1.35	3.69 ± 1.96	0.55 – 4.2	0.547
	Min-max	0.51 - 4.95	0.79 - 5.98		
FT_3 (pg/mL)	Mean ± SD	3.14 ± 1.96	2.96 ± 1.56	2.8 – 5.2	0.125
	Min-max	2.01 - 4.5	2.4 - 4.39		
FT_4 (ng/dL)	Mean ± SD	1.5 ± 0.65	1.7 ± 0.47	0.89-1.7	0.182
	Min-max	0.9 - 1.77	0.9 - 1.84		

*P significant if <0.05, **P highly significant if <0.001.

Serum ALT and serum total bilirubin values among the case and control groups were higher than the normal ranges. (Table 3**)**

Table (4): Comparison of Hematological Parameters, among the Case Group, before and after Treatment

Variable	Parameter	Before treatment	After treatment	p-value
Hb (g/dL)	Mean ± SD	6.86 ± 1.103	6.83 ± 1.109	0.894
	Min-max	5.10 - 8.70	5.40 - 8.90	
HCT (%)	Mean ± SD	22.74 ± 3.15	22.65 ± 3.137	0.888
	Min-max	15.9 - 25.9	15.9 - 24.7	
RBCs ($\times 10^6/\mu L$)	Mean ± SD	2.99 ± 0.54	3.10 ± 0.57	0.334
	Min-max	2.17 - 4.01	2.14 - 4.04	
MCV (fL)	Mean ± SD	69.6 ± 6.37	68.45 ± 6.34	0.377
	Min-max	58.4 - 80	58.7 - 81.3	
MCH (pg)	Mean ± SD	23.135 ± 3.78	23.29 ± 3.6	0.837
	Min-max	17.6 - 28.4	17.4 - 29.5	
MCHC (g/dL)	Mean ± SD	31.63 ± 1.15	31.51 ± 0.96	0.580
	Min-max	30 - 33.4	30 - 33.1	

PLT ($\times 10^9$/L)	Mean ± SD	418.29 ± 137.7	417.35 ± 122.54	0.971
	Min-max	180 - 602	179 - 630	
WBCs ($\times 10^9$/L)	Mean ± SD	10.21 ± 5.25	9.95 ± 3.74	0.780
	Min-max	3.2 - 21.7	3.3 - 18	
Ferritin (ng/mL)	Mean ± SD	1226.96 ± 453.38	873.63 ± 144.32	<0.001
	Min-max	760 - 1981	750 - 1150	

*P significant if <0.05, **P highly significant if <0.001.

The post-treatment values of Hb, HCT, RBCs, MCV, MCH, MCHC, PLT, and WBCs, among the case group, showed no statistically significant differences in comparison to the pre-treatment values (P>0.05), while serum ferritin values showed a statistically highly significant decrease (P<0.001) (Table 4).

Table (5): Comparison of Organ-Function Parameters among the Case Group, before and after Treatment

Variable	Parameter	Before treatment	After treatment	p-value
Urea (mg/dL)	Mean ± SD	24.81 ± 6.18	21.81 ± 3.91	0.009
	Min-max	18 - 37	15 - 30	
Creatinine (mg/dL)	Mean ± SD	0.688 ± 0.147	0.59 ± 0.102	0.001
	Min-max	0.49 - 0.92	0.4 - 0.8	
ACR (mg/g)	Mean ± SD	12.35 ± 6.43	12.58 ± 2.5	0.973
	Min-max	0.94 - 22.6	1.01 - 20.2	
ALT (U/L)	Mean ± SD	59.84 ± 17.23	52.7 ± 13.48	0.026
	Min-max	30 - 89	21 - 70	
AST (U/L)	Mean ± SD	31.92 ± 9.56	27.06 ± 6.99	0.006
	Min-max	19 - 52	18 - 40	

*P significant if <0.05, **P highly significant if <0.001. ACR: Albumin-to-Creatinine Ratio, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

The post-treatment values of blood urea, serum creatinine, serum ALT, and serum AST were statistically significant lower than the pre-treatment values (p<0.05), while post-treatment values of urinary ACR showed no statistically significant difference in comparison to the pre-treatment values (p>0.05) (Table 5)

Table (6): Comparison of the Total Antioxidant Capacity, Total Oxidant Status, and Oxidative Stress Index among the Study Groups, before and after Treatment

Variable	Parameter	Before treatment		After treatment		P1	P2	P3
		Case group n=48	Control group n=40	Case group n=48	Control group n=40			
TAC (mmol Trolox Equiv./L)	Mean ± SD	0.317 ± 0.089	0.324 ± 0.078	0.652 ± 0.129	0.294 ± 0.074	<0.001	0.081	<0.001
	Min-max	0.17 - 0.49	0.19 - 0.45	0.46 - 0.92	0.17 - 0.41			
TOS (µmol H_2O_2 Equiv./L)	Mean ± SD	49.31 ± 4.60	49.50 ± 7.37	37.14 ± 4.26	53.77 ± 7.94	<0.001	0.012	<0.001
	Min-max	38 - 59	41 - 65	30 - 47	44 - 70			
OSI	Mean ± SD	0.170 ± 0.055	0.168 ± 0.075	0.058 ± 0.012	0.204 ± 0.094	<0.001	0.063	<0.001
	Min-max	0.093 - 0.294	0.090 - 0.342	0.032 - 0.089	0.107- 0.411			

*P significant if <0.05, **P highly significant if <0.001. P1: significance among case group before and after treatment, P2: significance among control group at baseline and after 3 months, P3: significance among case and control groups after treatment.

At baseline, the mean TAC values were lower than the normal range, while the mean TOS values were higher than the normal range among the case and control groups.
The post-treatment mean values of TAC, TOS, and OSI showed statistically highly significant differences between the case and control groups ($p<0.001$).

Among the case group, the post-treatment mean values of TAC, TOS, and OSI showed statistically highly significant improvements in comparison to the control group and the pre-treatment values ($P<0.001$), while among the control group, the mean values of TAC and OSI showed no statistically significant differences at baseline and after 3 months ($P>0.05$), but the mean values of TOS, after 3 months, showed a statistically significant increase ($P<0.05$) (Table 6, Figure 1-3)

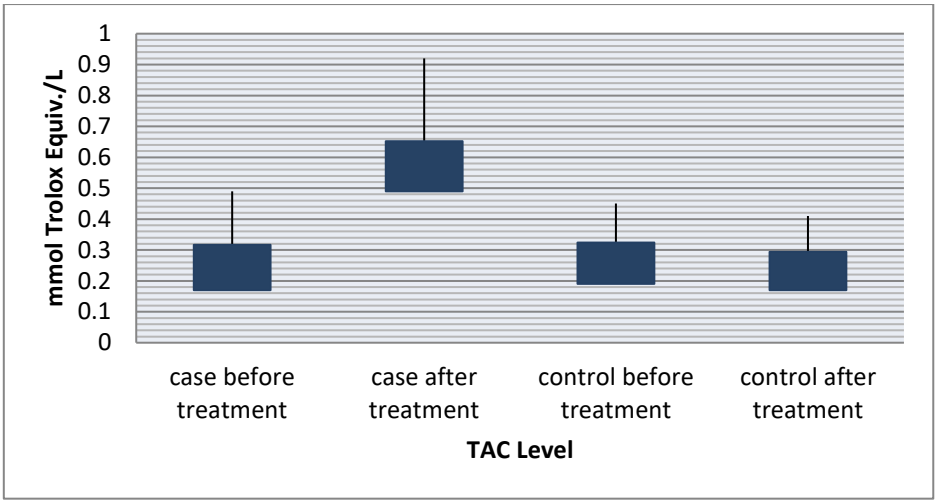

Figure (1): Total antioxidant capacity among the study groups, before and after treatment.

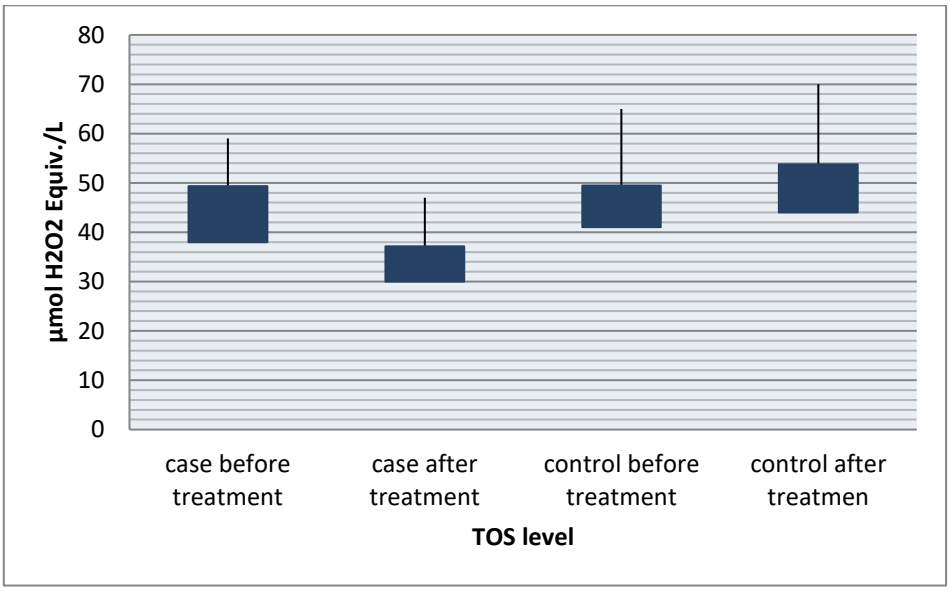

Figure (2): Total oxidant status among the study groups, before and after treatment

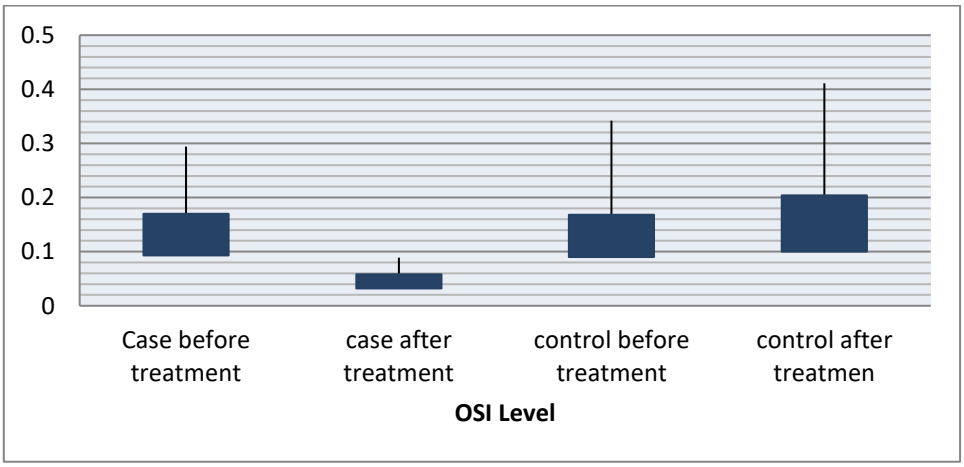

Figure (3): Oxidative stress index among the study groups, before and after treatment.

Discussion

Oxidative stress plays a major role in the pathophysiology of TDT patients. Regular blood transfusions as the standard treatment of TDT causes iron accumulation and peroxidative tissue injury. Consequently, it enhances oxidative stress in these patients, which may surpass the antioxidant defense capacity[12].

This study was conducted to evaluate the impact of a three-month antioxidant therapy regimen, consisting of vitamin E and NAC, on oxidative balance in TDT patients. The primary aim was to evaluate changes in oxidative stress biomarkers—TAC, TOS, and OSI—alongside secondary outcomes, such as hematological parameters, serum ferritin and organ-function parameters. It included 88 TDT patients, aged 5 - 18 years, divided into a case group (n=48) receiving antioxidants alongside the standard treatment and a control group (n=40) receiving only the standard treatment.

The demographic and clinical data of the study groups showed no significant differences as regards age, sex, weight, height, liver span, splenomegaly, and splenectomy between the case and control groups (P>0.05), denoting that both groups were properly cross matched (Table 1).

At baseline, the hematological parameters among the study groups, showed that mean values of Hb, HCT, RBCs, MCV, and MCH were lower than the normal ranges, while the mean values of MCHC, PLT, and WBCs were within the normal ranges, but the mean values of serum ferritin were higher than the normal range. However, no significant differences were observed between the case and control groups for any of the measured parameters (P>0.05) (Table 2).

This is in agreement with the studies done by Khawaji et al., 2020[12] and Karim et al., 2016[13], who found that Hb, HCT, MCV, and MCH have been significantly reduced in Beta-thalassemia patients except MCHC.

Also, Khawaji et al., 2020[12], and Ibrahim et al., 2023[14], observed a significant increase in serum iron and ferritin levels in Beta-thalassemia patients.

At baseline, the renal function parameters among the study groups showed that the mean values of blood urea, serum creatinine and urinary albumin/creatinine ratio were within the normal ranges. However, no significant differences were observed between the case and control groups for any of the measured parameters (P>0.05) (Table 3).

In concordance with our study results, Sen et al., 2015[15], did not find a significant difference in the serum urea, creatinine, or serum and urinary electrolytes (Na, K, Ca, and Ph) levels between Beta-thalassemia patients and controls, aside from a higher urinary protein/creatinine ratio in Beta-thalassemia patients.

On the other hand, Mahmoud et al., 2021[16], reported a significant increase in serum urea, creatinine, and K in Beta-thalassemia patients compared to the controls.

Again, Shaalan et al., 2022[17], found that the BUN level and the urinary Na/Cr, K/Cr, Ca/Cr, Mg/Cr, Ph/Cr, and albumin/creatinine ratios were significantly higher in the Beta-thalassemia patients than in the controls.

Discrepancies may reflect differences in patient populations.

At baseline, the liver function parameters among the study groups showed that the

mean values of ALT and total bilirubin were higher than the normal ranges, while AST and direct bilirubin were within the normal ranges. However, no significant differences were observed between the case and control groups for any of the measured parameters (P>0.05) (Table 3).

Our results agree with the studies done by Karim et al., 2016[13], Ibrahim et al., 2023[14], Attia et al., 2011[18], who found significantly higher ALT, AST, and ALP in Beta-thalassemia patients compared to healthy individuals.

Again, Verma et al., 2013[19], reported that an elevated serum total bilirubin and indirect bilirubin were observed among β-thalassemia patients.

At baseline, the thyroid function parameters among the study groups showed that the mean values of TSH, FT3, and FT4 were within the normal ranges with no significant differences were observed between the case and control groups (P>0.05) (Table 3).

Align with our results, Karim et al., 2016[13], observed no significant differences in thyroid function parameters among Beta-thalassemia patients compared to healthy individuals.

Also, Aleem et al., 2000[20], demonstrated that hypothyroidism is primarily a disease of the second decade of life.

At baseline, the mean values of random blood glucose were within the normal range with no significant difference were observed between the case and control groups (P>0.05) (Table 3).

In agreement with our results, Metwalley and El-Saied, 2014[21], found that the mean values of fasting plasma glucose (106.7±33.1 mg/dL) and 2-hour post-load plasma glucose (139.6±78.3 mg/dL) among Beta-thalassemic patients, while the prevalence of diabetes was 5% (3 of 60) and IGT was 8% (5 of 60).

Post-treatment, the case group exhibited no significant changes in Hb, HCT, RBCs indices, PLT, and WBCs (P>0.05), while serum ferritin showed a highly significant reduction compared to the pre-treatment values (P<0.001) (Table 4).

In line with our results, Mohamed et al., 2020[22], observed a significant difference in serum ferritin after a 3-month NAC supplementation, this result reflecting an improvement in the oxidative balance.

Parallel with our results, Pfeifer et al., 2009[23], Fibach and Rachmilewitz, 2010[24], reported a significant impact of short-term consumption of vitamin E on the improvement of oxidative stress but not clinically on anemia, both in Beta-thalassemia intermedia and TDT.

Fibach and Rachmilewitz, 2010[24], explained that by, as the duration of RBCs turnover is about 100 days, it takes a longer duration of treatment to influence RBCs' turnover and hemoglobin levels.

Also, Haghpanah et al., 2021[25], observed that hemoglobin levels did not significantly differ at the end of the study in TDT patients who were taking vitamin E and NAC.

On the other hand, Yanpanitch et al., 2015[26], reported improvements in hemoglobin, iron overload, oxidative stress, and hypercoagulable state in patients with Beta-thalassemia/hemoglobin E who received a combination of vitamin E and NAC and an iron chelator (DFP).

Discrepancies may reflect differences in patient populations (e.g. Beta-thalassemia/hemoglobin E vs TDT or coadministration of DFP).

Post-treatment, the case group demonstrated significant improvements in

the blood urea, serum creatinine, serum ALT, and serum AST (P<0.05), while urinary ACR showed no significant difference compared to the pre-treatment values (P>0.05) (Table 5).

Significant reduction in serum ferritin and improvements in organ function suggest that antioxidant therapy may enhance chelation efficacy or protect against iron-induced oxidative damage.

At baseline, the mean values of TAC were lower than the normal range, while the mean values of TOS were higher than the normal range among the case and control groups. However, no significant differences were observed between the case and control groups (P>0.05).

In line with our study results, Ibrahim et al., 2023[14], Metwalley and El-Saied, 2014[21], Mohamed et al., 2020[22], Kassim et al., 2024[27], Khail et al., 2020[28], found that the values of TAC were significantly lower and the values of TOS and MDA were significantly higher in Beta-thalassemia patients than controls**.**

Post-treatment, the case group exhibited a highly significant increase in TAC, decrease in TOS and reduction in OSI compared to the pre-treatment values and the control group (P<0.001), while the control group showed no significant changes in TAC and OSI, but TOS showed a significant increase compared to that at baseline (p>0.05). (Table 6 and Figure 1-3).

In concordance with our study results, Mohamed et al., 2020[22], found that a 10 mg/kg/day of NAC for 3 months demonstrated a significant improvement in oxidative biomarkers, TOS values showed a significant decrease (P<0.001), TAC values showed a significant increase (P<0.001), and OSI values showed a remarkable reduction (p<0.001).

Also, in a randomized controlled trial, by Khail et al., 2020[28], the TOS of 44 children with Beta-thalassemia major, a mean age of 8.05±3.45 years, was assessed for a 3-month-treatment with 10 mg/kg/day of NAC. The study exhibited a significant decreased in TOS level (p<0.001).

Again, Ozdmir et al., 2014[29], reported a significant decrease in TOS and a significant increase in TAC and hemoglobin levels after 3 months of treatment with NAC in children with Beta-thalassemia major.

Conclusion

- A three-month regimen of vitamin E (10 mg/kg/day) and NAC (10 mg/kg/day) significantly improved oxidative damage in TDT patients, as evidenced by increased TAC, reduced TOS and OSI, lower serum ferritin, and better renal and liver functions. While it does not directly ameliorate anemia, the therapy offers a protective effect against oxidative damage, supporting its potential inclusion in TDT patients' management protocols.

Recommendations

- Antioxidant therapy with vitamin E and N-acetylcysteine should be included in TDT patients' management protocols.
- A further multicenter case-control research including a larger sample size and a long-term follow up using combinations of antioxidants in TDT patients to study

their clinical and biomarkers outcomes.

Limitation of the Study

- With only 88 participants, the study may lack the statistical power to detect small to moderate effects, particularly regarding the heterogeneity of TDT presentations.
- The 3-month treatment period may be insufficient to observe long-term benefits or changes in chronic conditions associated with TDT.
- The study did not follow patients over a longer duration, which could have provided insight into the sustainability of any observed benefits of antioxidant therapy.

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