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Original Article

Relationship between Oxidative Stress and the Blood Iron Concentration and Antioxidant Status in Major ß-thalassemia in Iraq

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Abstract

Reduction or total lack of beta-globin chains caused by a congenital disease called ß-thalassemia major is one of the lives threatening diseases. Patients who suffer from ß-thalassemia need a repeated blood transfusion for survival. The repeated blood transfusion in ß-thalassemia patients may cause oxidative stress and tissue injury due to iron overload, altered antioxidant enzymes, and other essential trace element levels. The current study aimed to investigate the correlation of oxidative stress with serum trace element levels and antioxidant enzyme status in ß-thalassemia major patients. A total of 130 serum samples were obtained from ß-thalassemia major patients (n=100; 50 males and 50 females) and healthy individuals (n=30; 15 males and 15 females). Hematological parameters were measured on both groups by a comprehensive blood test that included the amount of hemoglobin Hb, packed cells volume, number of red blood cells, mean corpuscular volume ratio, mean corpuscular hemoglobin ratio, mean corpuscular hemoglobin concentration, red cell distribution width, white blood cells, and platelets counts. All of these blood parameters showed a clear decrease in thalassemia patients, except for red blood cells and platelets counts, which demonstrated a significant increase. The highest significant mean for iron in males and females were 233.768 and 219.150 µgm\dL in patients, respectively, while the mean level of iron significantly reduced in the control group (113.40 and 103.33 µgm\dL in males and females, respectively). The results indicated a significant decrease in uric acid in males and females in the patient group (41.042 and 40.582 mg\L in males and females, respectively), compared to the control group (53.866 and 43.60 mg\L in males and females, respectively). Allantoin concentration was detected by high-performance liquid chromatography technique, the results of which showed that the highest values in patients were 62.822 and 25.480 mg\L in males and females, respectively, compared to the control group 2.342 and 1.481 mg\L in males and females, respectively. Superoxide dismutase concentration decreased in patients (129.635 and 111.848 U\mL in males and females, respectively), compared to the control group (208.623 and 190.413 U\ml in males and females, respectively).

Keywords: Antioxidant, Beta-thalassemia, Superoxide dismutase, Trace elements, Uric acid\Allantoin

1. Introduction

Mediterranean anemia (thalassemia) is an inherited hemoglobinopathy caused by a defect in the formation of globin chains (1). This disease is characterized by the production of an insufficient amount of globin chains (Alpha, Beta, or both), and depending on the type of affected globin chain, the anemia can be classified as the following types: Mediterranean and Mediterranean type Alpha and Beta. Mediterranean anemia is a consequence of reduced or absent production of the Beta-globin chain which is a result of a mutation in the Beta-globin gene located on the chromosome (11), and if this mutation occurs in the Beta-globin gene, the disease is called Mediterranean anemia. In the studies conducted on

patients with Mediterranean anemia in Babylon Province, Iraq, it was noted that most of the patients belonged to the Beta-medial anemia category and one patient belonged to the β-aplastic group. Therefore, that study was conducted on patients with Beta-mediated anemia only. According to the statistics, the Ministry of Health of Iraq showed that the number of people with Mediterranean anemia has reached 15,000 (395 patients in Babylon Province, of whom 283 cases were diagnosed with Mediterranean great Beta anemia). Although Mediterranean major anemia is the Beta type of a common genetic disease, it is the most severe type of disease. However, scarce studies have been conducted on the hematological variables, biochemical changes, and oxidative stress. Moreover, few studies have been dedicated to investigating the existence of allantoin in affected patients serum by high-performance liquid chromatography (HPLC) technique that occurs in patients with this disease in Babylon Province. The current study aimed to investigate the relationship between some trace elements, such as Iron, in the blood serum of patients with Beta-thalassemia and their relationship with non-enzymatic antioxidants, such as some organic molecules (Uric acid and Allantoin), and determine total antioxidant capacity (TAC) and enzyme activity, such as superoxide dismutase (SOD). In addition, it was conducted to prepare allantoin from uric acid in the laboratory and configure the standard curve for different concentrations of allantoin, and afterward, measure the allantoin level in the serum samples of patients and compare it with that in the control group using the HPLC technique.

2. Materials and Methods

2.1. Experimental Design

The research was carried out in the Organic Chemistry and Animal Physiology Laboratory for Postgraduate Studies in the College of Science for Women, Department of Chemistry and Biology in Babylon University, Iraq, in cooperation with the Thalassemia Center (Genetic Blood Diseases) and the laboratories of Al-Hilla Teaching Hospital of the Maternity and Children, Iraq. It was confirmed that the patients had thalassemia major through periodically reviewing them in the Thalassemia Center and being diagnosed by a specialist with the severe deficiency of blood parameters (amount of hemoglobin, the packed cells volume, the number of red blood cells, mean corpuscular volume ratio, mean corpuscular hemoglobin ratio, mean corpuscular hemoglobin concentration, and red cell distribution width).

Blood samples were collected from patients with Beta-Mediterranean anemia who attended the Thalassemia Center to obtain a blood transfusion, and their disease was diagnosed in advance. These patients suffered from an excess of iron in their bodies due to the blood transfusion, which led to some complications, including chronic hepatitis and an enlarged spleen. A total of 130 serum samples was obtained from ß-thalassemia major patients (n=100; 50 males and 50 females) and healthy individuals (n=30; 15 males and 15 females) at the age range of 1-40 years old within November 2020-June 2021.

2.2. Blood Samples

An amount of 4 ml of venous blood was taken from each patient before performing a transfusion. The same blood volume of venous blood was obtained from healthy people as the control group. The samples were divided into two parts as follows:

1- An amount of 1 ml of blood was added into an ethylenediamine tetraacetic acid tube, for haematological assay.

2- A volume of 3 ml of blood was added in a gel tube for serum separation, and was used for biochemical assays. The sample was centrifuged at 3,000 rpm for 5 min; as a result, approximately 2 ml of serum was separated and used for the biochemical assays.

2.3. Iron Direct Method (Ferene)

After dissociation of iron-transferrin bound in acid medium, ascorbic acid reduced Fe into Fe^{+2}. Fe^{+2} then formed a colored complex with 3-(2-Pyridyl) -5, -6-difuryl-1, -2, 4-triazine-disulfonate (Ferene). The absorbance was measured at 600 nm (580-620). It was directly related to the amount of iron in the specimen.

Thiourea was added to the reagent to prevent copper interference. Reagent compositions for this assay are repressed in table 1.

Table 1. Reagent compositions

Vial R1 (Reductant)	
Citric acid	150 mmole/L
Ascorbic acid	30 mmole/L
Thiourea	27 mmole/L
Vial R2 (Chromogen)	
Ferene	600 µmole/L
Vial R3 Standard	
Iron	200 µg/dl (35.8 µmole/L)

2.3.1. Reagent Preparation

The working reagent was prepared by mixing RI (50 volumes) and R2 (1 volume). Cleaned material was used carefully with 0.1 N of HCI and well rinsed with distilled water. Special care was given to the quality of water, reagents, and/or specimens. Some automated instruments required special preparation. The stand reagents and specimens were kept at room temperature. The sets of tubes were prepared according to the following boards (Table 2).

The result was calculated as follows:

Result = (A2-A1)Assay/(A2-A1)Standard × Standard concentration

Table 2. Sets of tubes

Blank tubes	**Blank**	**Standard**	**Assay**
Reagent R1	1 mL	1 mL	1 mL
Specimen			200 µL
Standard		200 µL	
Distilled water	200 µL		
Mix gently, let stand for at least 3 min at room temperature. Record A1 absorbance at 600 nm (580-020) against blank. The color was stable for 1 h.			
Assay tubes	**Blank**	**Standard**	**Assay**
Working reagent	1 mL	1 mL	1 mL
Specimen			200 µL
Standard		200 µL	
Distilled water	200 µL		
Mix gently, let stand for at least 5 min at room temperature. Record A2 absorbance at 600 nm (580-020) against blank. The color was stable for 1 h.			

Note. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

2.4. Uric Acid (Uricase method)

Uricase acts on uric acid to produce allantoin, carbon dioxide, and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase reacts with chromogen (amino-antipyrine and dichloro-hydroxybenzensulfonate) to yield quinoneimine, a red-colored complex. The absorbance was measured at 520 nm (490-530) and was directly related to the amount of uric acid in the specimen. Reagent compositions for Vial R1 enzymes are provided in table 3. The contents of vial R1 (Enzymes) were added promptly into vial R2 (Buffer) and were mixed gently up to complete dissolution before using reagent (approximately 2 min). The reagent and specimens were kept at room temperature. In this study, the uricase method was used as described by Liao, Zhao (2).

Table 3. Enzymes

Vial R1 Enzymes	
Potassium hexacyanoferrate (II)	42 µmole/L
Peroxidase	≥450 U/L
Amino-antipyrine	0.150 mmole/L
Uricase	≥120 U/L
Vial R2 Buffer	
Dichlorohydroxybenzensulfonate	2 umol/L
Tris pH 8.0 at 25°C preservative	50 umol/L
Vial R3 Standard	
Uric acid	10 mg/dL (595 umol/L)

Notes. 1. Serum, plasma, or urines was diluted (1+9) with demineralized water. **2.** Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support. **3.** Specimen: a 20 ul volume may be used (increased linearity, however, slightly decreased sensitivity).

2.5. Total Superoxide Dismutase Activity Assay Kit (Hydroxylamine Method)

This kit can be used to measure total superoxide dismutase (T-SOD) activity in serum, plasma, urine, cells, cell culture supernatant, and tissue samples. The superoxide anion-free radical ($O_2^{\bullet-}$) can be produced by xanthine and xanthine oxidase reaction system; it oxidizes hydroxylamine to form nitrite and turn to purple under the reaction of the developer. When the measured samples contain SOD, the SOD

can specifically inhibit superoxide anion-free radical (O2•-). The inhibitory effect of SOD can reduce the formation of nitrite; the absorbance value of the sample tube is lower than the control tube. The SOD of the sample was calculated according to the computational formula. Table 4 shows the T-SOD activity assay kit (Elabscience, Iraq, Cat.No.:E-BC-K019-S) components and storage. The T-SOD activity assay kit was used as described by Elabscience, USA.

Table 4. Kit components and storage

Item	Component specification storage	Component specification storage	Component specification storage
Reagent 1	Buffer solution	12 mL×1 vial	2-8C, 6 months
Reagent 2	Nitrosogenic agent	12 mL×1 vial	2-8℃, 6 months
Reagent 3	Substrate solution	12 mL×1 vial	2-8℃, 6 months
Reagent 4	Enzyme stock solution	0.6 mL×1 vial	-20℃, 6 months
Reagent 5	Enzyme diluent	12 mL×1 vial	2-8℃, 6 months
Reagent 6	Chromogenic agent A	Powder×1 vial	2-8℃, 6 months
Reagent 7	Chromogenic agent B	Powder×1 vial	2-8℃, 6 months
Reagent 8	Chromogenic agent C	60 mL×1 vial	2-8℃, 6 months

Note. The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed.

2.6. Separation of Allantoin from Uric Acid

The procedure for allantoin separation was previously described by Pizzichini, Arezzini (3).

2.7. Determination of Allantoin Level Concentration in Serum by High-Performance Liquid Chromatography Technique

1) Sample Preparation

A serum sample of 100 µm was mixed with 400 µm of solvent C (KH2PO4/H3PO4-buffer with 50 mmol/1 phosphate, pH 4.60), and then filtered through a membrane filter with a pore diameter of 0.22 µm (Germany). An aliquot (50 µm) of the filtrate was directly injected into the injector of the HPLC device. The quantification is based on the peak areas calculated for the wavelength (4).

2) Preparation of Standard Solutions

An amount of 2 mg of each standard was taken and placed in a volumetric flask (25 ml) and the volume was supplemented with methanol (HPLC 99.9 %) up to the stock solution concentration (80 ppm). Using the dilution law of C 1 V 1 = C 2 V 2, the concentrations injected into the HPLC were prepared.

3) High-Performance Liquid Chromatography Condition

The HPLC (SYKAMN, Germany) was used to analyze, add, and detect vincristine and vinblastine. The mobile phase was an isocratic acetonitrile-0.1 M phosphate buffer containing 0.5% glacial acetic acid (30:70) at a flow rate of 1.2 mL/min, the column was C18-ODS (25 cm×4.6 mm) and the detector ultraviolet-360 nm (4).

2.8. Statistical Analysis

Data were tested for normal distribution by graphic inspection of the residues and homogeneity of variances using Levene's test. This test confirmed that the use of a parametric test was appropriate for subsequent analyses. Therefore, the t-test was employed to compare the values of the beta-thalassemia and control groups. Statistical analyses were carried out in the Statistica 7 software (Statsoft Inc.) and the level of significance was set at 5% (P<0.05).

3. Results and Discussion

3.1. Iron Level in Serum Samples

Table 5 and figure 1 show that the highest significant mean for iron in males and females were 233.768 and 219.150 µgm\dL, respectively, while the mean level of iron significantly reduced in the control group (113.40 and 103.33 µgm\dL for males and females, respectively). There was a highly significant difference between the studied groups ($P \leq 0.05$). Regarding differences between genders in each group, the results indicated non-significant differences between genders in the control and patient groups.

Table 5. Concentration level of iron in β-thalassemia patients (males and females) and control group

Statistical standards	Study groups				*P-value
	Patients (n=100) µgm\dL		Control (n=30) gm\dLµ		
	Male (n=50)	Female (n=50)	Male (n=15)	Female (n=15)	
Mean	233.768	219.150	113.40	103.33	
Standard deviation	169.919	89.907	39.039	40.163	
Minimum	63.414	63.414	65.00	50.000	0.000
Maximum	598.947	363.550	175.00	170.000	(H.S.)
Standard error	24.03	12.714	10.08	10.37	
*P-value	0.592 (N.S.)		0.492 (N.S.)		

*t-test, H.S.: Highly significance; Sig: Significance, N.S.: No significance: *P*-value ≤ 0.05

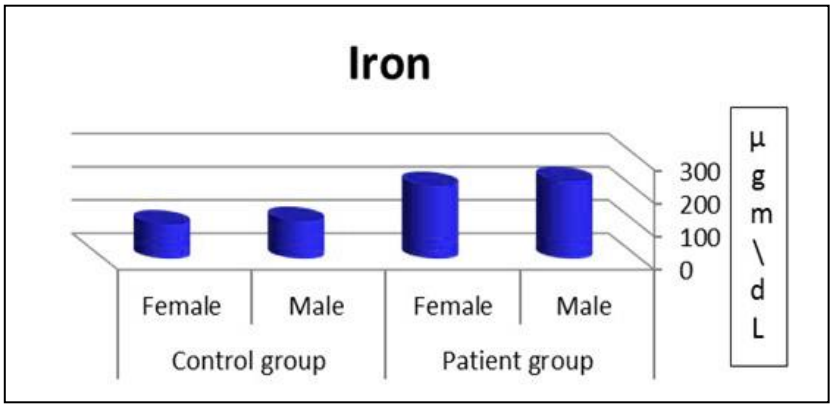


Figure 1. Concentration of iron in studied groups (males and females) among thalassemia patients and control group

Patients with β-thalassemia are mainly exposed to oxidative stress due to iron overload (through the Fenton reaction). Therefore, the evaluation and maintenance of antioxidant defense can be useful in protecting β-thalassemia patients from more serious complications of the disease (5).

$Fe^{2+} + H2O2$ ——— ÓH+ ¯OH + Fe^{3+} ----- (1

The results of the present study were consistent with those of studies conducted by Tracz, Alam (6), Al-Samarrai, Adaay (7), Piga, Longo (8), Kuppusamy and Tan (9), and Abdulla (10) that revealed the increase of iron concentration in serum patients. The findings some studies have reported the elevated level of iron attributed to hemochromatosis is caused by an excess of iron in the body, which can be either primary or secondary (9, 11, 12). Primary hemochromatosis is a condition in which the body's iron stores are depleted. Increased iron absorption is a symptom of a hereditary disease. As a result, the body is overloaded with iron. Secondary, hemochromatosis develops due to such

disorders as thalassemia. Iron overload is common in thalassemia major, where it occurs on a regular basis. There will be a need for blood transfusions. Major beta-thalassemia patients require frequent blood transfusions, which can result in complications. In the absence of adequate chelation therapy, iron overload can occur.

This iron accumulation in thalassemia individuals might exceed ferritin storage and detoxifying capability, entirely saturating transferrin and resulting in the generation of free iron in the blood and tissues. The development of very damaging chemicals, such as hydroxyl radicals, will be caused by these free iron hydroxyl radicals which are extremely reactive, attack lipids, and form lipid peroxides, which contribute to oxidative stress (13, 14).

The blood transfusion is performed every 2-4 weeks to treated severe anemia, which results in iron overload in various tissue, including the liver, heart, and endocrine tissue. The kidneys are another site of iron accumulation in thalassemia, unlike in the other organs; it is unclear whether kidney infection results solely from intravascular hemolysis, chronic transfusion, or as a complication of iron chelation therapy (6).

The significant increase of serum iron and ferritin in Iraqi patients indicated an existing iron overload. The repeated transfusion of blood causes iron overload in the β-thalassemia without chelation therapy that leads to numerous complications, such as splenomegaly and influence on liver function (8). Nevertheless, if free iron was available, it reacted with H_2O_2 to form hydroxyl radicals that were extremely reactive species leading to depolymerization of polysaccharide, DNA strand breakage, and inactivation of functional proteins (9, 11-13).

3.2. Level of Uric Acid in Serum Samples

Table 6 and figure 2 demonstrate a significant decrease in uric acid in males and females in the patient group (41.042 and 40.582 mg\L in males and females, respectively), compared to the control group (53.866 and 43.60 mg\L in males and females, respectively). There was a significant difference between studied groups at the p-value of ≤ 0.05). Regarding the differences between genders in each group, the results showed significant differences between genders in the control group, while there were non-significant differences between genders in the patient group.

Table 6. Concentration level of uric acid in β-thalassemia patients (males and females) and control group

Statistical Standards	Study groups				*P-value
	Patient group (n=100) (mg\L)		Control group (n=30) (mg\L)		
	Male (n=50)	Female (n=50)	Male (n=15)	Female (n=15)	
Mean	41.042	40.582	53.866	43.60	
Standard deviation	11.389	16.057	11.525	11.037	
Minimum	22.05	20.62	36.00	26.00	0.006
Maximum	78.00	76.00	72.00	60.00	(Sig)
Standard error	1.61	2.27	2.975	2.849	
*P-value	0.869 (N.S.)		0.019 (Sig)		

*t-test; Sig: Significance; N.S.: No significance; *P*-value ≤ 0.05

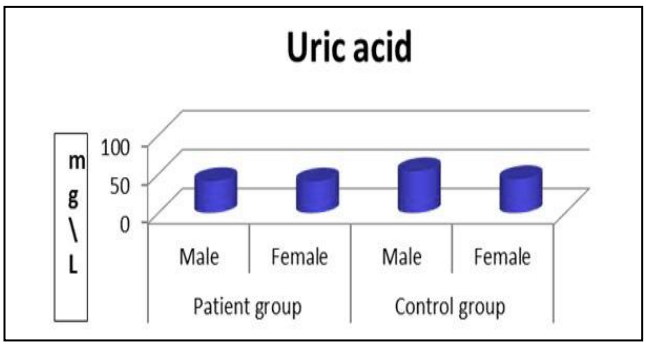


Figure 2. Concentration uric acid in studied groups (males and females) among thalassemia patients and control group

3.3. Level of Allantoin in Serum Samples

Table 7 and figure 3 show that the allantoin concentration was detected by HPLC technique, and the results indicated the highest values in patients (62.822 and 25.480 mg\L in males and females, respectively), compared to the control group (2.342 and 1.481 mg\L in males and female, respectively). There was a highly significant difference between the studied groups ($P \leq 0.05$). Regarding the differences between genders in each group, the results showed highly significant differences between genders in the patient group, while there were non-significant differences between genders in the control group.

3.4. Estimation of Scavenger System

The uric acid-allantoin pathway is generated in the human body by the oxidation of purines; however, no enzyme is present to oxidize it further. The levels of uric acid significantly reduced in patients (male and female), compared to the corresponding levels in the control group (Table 6). The levels of allantoin significantly increased in patients (male and female), in comparison to the corresponding levels in control individuals (Table 7). There was a nonsignificant correlation between Allantoin/Uric acid ratio (r=-0.105) as shown in figure 4.

This observation is of great importance since it demonstrated that the scavenger system was greatly depleted with the generation of reactive nitrogen species and ROS in thalassemia patients (males and females), and it indicated that ROS was the major contributor of oxidative stress in male thalassemia patients than their female counterparts. Since the estimation of the uric acid-allantoin pathway was conducted for the first time in thalassemia patients, to the best of our knowledge, the literature concerning this topic is completely unavailable. Uric acid is a known water-soluble scavenger that proved to exert an important antioxidant activity (18). It is noteworthy to mention the specifications of the metabolic pathway of uric acid. First, the synthesis of uric acid is conducted via the activation of xanthine dehydrogenase under physiological conditions. In pathological conditions (particularly oxidative stress), the xanthine oxidase enzyme is activated by ROS leading to the formation of uric acid. Therefore, most probably, the uric acid level in this study was related to the activation of the xanthine oxidase enzyme. Second, in human beings, the responsible enzyme for uric acid degradation is uricase which is not available under physiological conditions. This enzyme and its end product from uric acid, notably allantoin, is not detected in biological fluids (18). Consequently, the results of this research showed that the oxidative stress in thalassemia patients was counteracted by the uric acid-allantoin pathway as it was reflected by the depletion of uric acid and increase in allantoin level. Further analysis revealed that the activity of the uricase-like enzyme was enhanced in thalassemia patients by 3-fold of that of controls.

Table 7. Concentration level of allantoin in β-thalassemia patients (males and females) and control group

Statistical standards	Study groups				*P-value
	Patient group (n=60) (mg\L)		Control group (n=20) (mg\L)		
	Male (n=30)	Female (n=30)	Male (n=10)	Female (n=10)	
Mean	64.106	26.206	2.448	1.477	0.000 (H.S.)
Standard deviation	13.421	6.266	1.390	0.883	
Minimum	48.8	17.6	0.00	0.00	
Maximum	80.50	31.90	3.84	2.40	
Standard error	2.109	1.144	0.439	0.294	
*P-value	0.000 (H.S.)		0.131 (N.S.)		

*t-test; H.S.: highly significance; Sig: Significance; N.S.: No significance; *P*-value ≤ 0.05

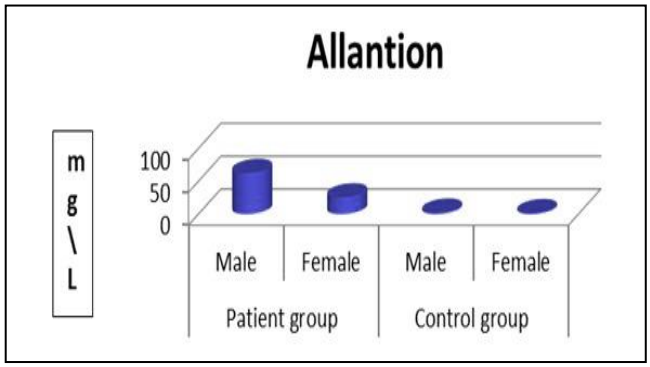
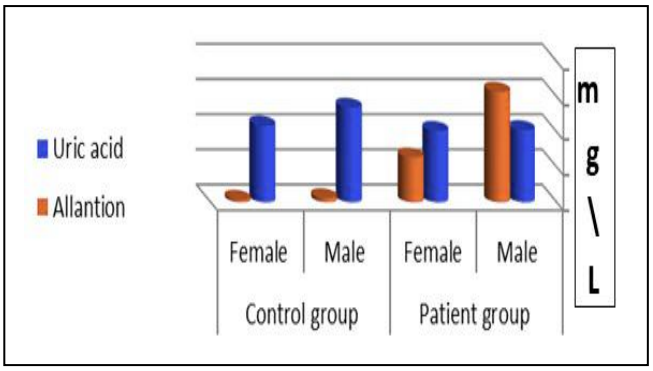


Figure 3. Concentration allantoin in studied groups (males and females) among thalassemia patients and control group



Figures 4. Relationship between uric acid and allantoin in studied groups (males and females) among thalassemia patients and control group

3.5. Total Antioxidant Capacity in Serum Samples

Table 8 and figure 5 show that the mean of TAC in males and females in the patient group were 11.287 and 6.678 U\mL, respectively, compared to that in the control group 85.660 and 80.660 U\mL, respectively. There were highly significant differences between studied groups at the p-value of ≤ 0.05. Regarding differences between genders in each group, the results revealed highly significant differences between genders in the patient group, while non-significant differences were observed between genders in the control group.

The depletion of TAC induced by oxidative stress is eliminated by the release and stock of organ antioxidants, mainly from the liver and adipose tissues, and the induction or activation of antioxidant enzymes. These findings were in line with those of our study and a study conducted by Lamia (13). Bata-thalassemia major and minor are characterized by an overproduction of free radicals; in other words, when the antioxidant defense of an organism is overwhelmed or established, a deficit occurs in the defenses of the organism against oxidation. The primary defense against oxidative stress in extracellular fluids results from a number of low molecular weight antioxidant molecules. These antioxidants can also be generated during normal metabolism (e.g., uric acid, bilirubin, albumin, and thiols) or introduced in the body by the consumption of dietary products rich in antioxidants (e.g., olive oil, fruits, vegetables, tea, and wine).

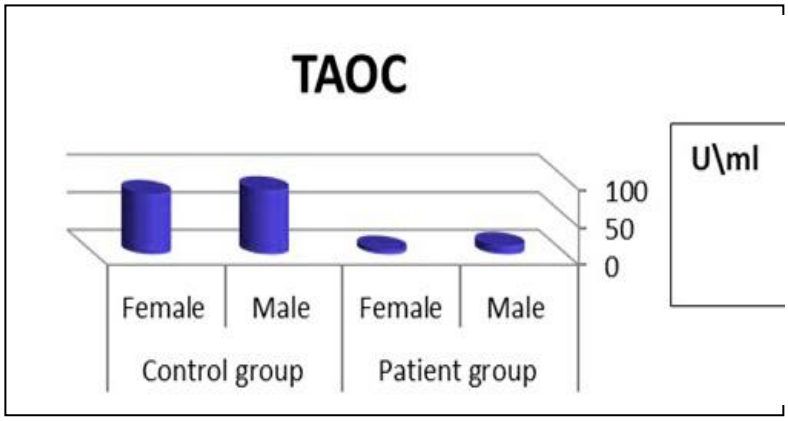
At a later phase of oxidative stress, TAC falls due to the depletion of antioxidants, and low molecular weight antioxidants penetrate specific locations in the cell where oxidative stress may occur and protect against free radicals. The sum of endogenous and food-derived

antioxidants represents the total antioxidant activity of the extracellular fluid. In addition, the levels of these antioxidants are suitable not only as a protection against oxidation but also reflect their consumption during acute oxidative stress states. The cooperation among different antioxidants provides greater protection against attack by reactive oxygen or nitrogen radicals than any single compound alone. Therefore, the overall antioxidant capacity may give more relevant biological information compared to that obtained by the measurement of individual parameters as it considers the cumulative effect of all antioxidants present in serum and body fluids.

Table 8. Concentration level of total antioxidant capacity in β-thalassemia patients (males and females) and control group

Statistical standards	Study groups				*P-value
	Patient group (n=100) (U\mL)		Control group (n=30) (U\mL)		
	Male (n=50)	Female (n=50)	Male (n=15)	Female (n=15)	
Mean	11.287	6.678	85.660	80.660	
Standard deviation	4.442	4.133	14.984	14.984	
Minimum	4.133	1.033	68.30	63.300	0.000
Maximum	21.843	16.001	121.30	116.30	(H.S.)
Standard error	2.109	1.144	0.439	0.294	
*P-value	0.000 (H.S.)		0.389 (N.S.)		

*t-test: H.S.: Highly significance: Sig: Significance; N.S.: No significance; *P*-value ≤ 0.05



Figures 5. Concentration of total antioxidant capacity in groups (males and females) among thalassemia patients and control

3.6. Level of superoxide dismutase in serum samples

Table 9 and figure 6 shows that SOD concentration decreased in patients (129.635 and 111.848 U\mL in males and females, respectively), compared to the control group (208.623 and 190.413 U\ml in males and females, respectively). There were highly significant differences between studied groups at the p-value of ≤ 0.05. Regarding differences between genders in each group, the results showed highly significant differences between genders in the control and patient group.

Table 9. Distribution of superoxide dismutase in studied groups

Statistical standards	Study groups				*P-value
	Patient group (n=100) U\mL		Control group (n=30) U\mL		
	Male (n=50)	Female (n=50)	Male (n=15)	Female (n=15)	
Mean	129.635	111.848	208.623	190.413	
Standard deviation	5.643	4.437	15.643	5.088	
Minimum	115.7	97.6	200.2	185.8	0.000
Maximum	151.0	161.1	233.4	200.1	(H.S.)
Standard error	0.798	0.627	4.039	1.313	
*P-value	0.000 (H.S.)		0.000 (H.S.)		

*t-test; H.S.: Highly significance: Sig: Significance; *P*-value ≤ 0.05

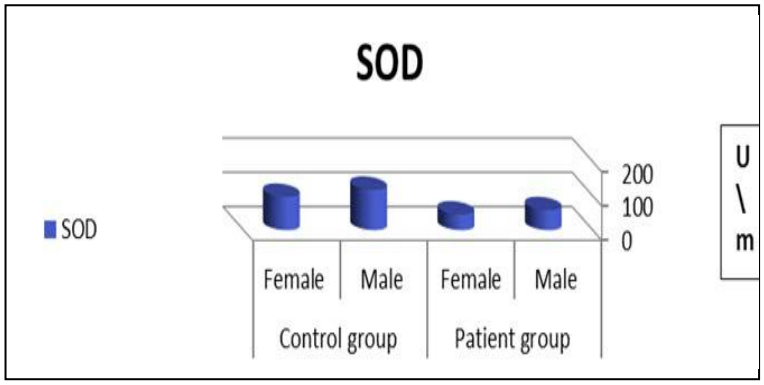


Figure 6. Concentration of superoxide dismutase in studied groups (males and females) among thalassemia patients and control group

Our results are in agreement with those conducted by Dhawan, KhR (19), who found that the mean SOD enzyme activity was at least 1.5 times lower in the thalassemia than in controls. The findings pertaining to SOD enzyme activity reported by other investigators are varied. They ranged from high SOD activity to no difference in patients and controls (20, 21).

The findings of our study were in line with those of a study performed by Patne, Hisalkar (22) examining 50 β-thalassemia major and 50 healthy controls and finding that serum SOD and glutathione activities significantly decreased in thalassemia patients, compared to healthy individuals. These results were also consistent with those of the study carried out by Waseem, Khemomal (23) who showed that the levels of these enzymes were significantly lower (P<0.001) in thalassemia patients than in control groups. The findings of a study conducted by Choudhary, Vyas (24) were in agreement with those of our study. The low activity of SOD in our study might suggest that a longer disease duration would lead to SOD induction, and consequently, progressive decrease in its activity since non-enzymatic glycation, the other cause of hydrogen peroxide production, later predominates and further inhibits Cu/Zn-SOD. The formation of hydrogen peroxide inactivates SOD (25). Therefore, the accumulation of hydrogen peroxide may be one of the explanations for decreased activity of SOD in these patients. The primary catalytic cellular defense that protects cells and tissues against potentially destructive reactions of superoxide radicals and their derivatives is the Cu/Zn-SOD. It has been observed that SOD can be rapidly induced in some conditions when cells or organisms are exposed to oxidative stress (10, 26).

Patients with thalassemia show various clinical signs and symptoms that seem to be unavoidable due to the regular transfusion of blood and its associated

physiological and pathological outcomes. The decreased level of uric acid in the current study for the group of patients, compared to the control group, might be due to the presence of free radicals (i.e., ROS) as uric acid interacts with it and generates from this oxidation to yield allantoin compound 1\3 (U\A). Allantoin concentrations were high for the group of thalassemia patients. Decreased TAC and SOD enzyme indicated multiple free radical formations as a result of thalassemia disease.

Authors' Contribution

Study concept and design: R. S. N. and I. A.
Acquisition of data: R. S. N. and I. A.
Analysis and interpretation of data: R. S. N. and I. A.
Drafting of the manuscript: R. S. N. and I. A.
Critical revision of the manuscript for important intellectual content: R. S. N. and I. A.
Statistical analysis: R. S. N. and I. A.
Administrative, technical, and material support: R. S. N. and I. A.

Ethics

All the procedures in this study, were performed according to the guidelines instructed by the Human Ethics Committee of the University of Babylon, Babylon, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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