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## Evaluation the Non enzymatic antioxidants in patients with $\beta$ -thalassemia major among Iraqi patients

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### KEY WORDS:

$\beta$ -Thalassemia major,  
Coenzyme Q10, vitamin C,  
Vitamin E, uric acid.

### ABSTRACT

**Background:**  $\beta$ -thalassemia major ( $\beta$ -TM) is a major health problem in the world that forces patients to repeat blood transfusions. Frequent blood transfusions cause toxic iron overload. The association between serum iron levels and  $\beta$ -thalassemia major has been studied extensively in the literature. Several studies have evaluated the oxidant and antioxidant statuses of thalassemia patients. However, most studies have focused mainly on the enzymatic antioxidant and intermediate states and thus few data are available for non-enzymatic antioxidant like Coenzyme Q10.

**aim of study:** To evaluate the concentration of some non- enzymatic antioxidant measurements like Coenzyme Q10, Vitamin C, Vitamin E, uric acid, and bilirubin in the plasma of patients who suffered from beta – thalassemia major.

**Patients and Methods:** Case- control study designed includes 90 participating (60  $\beta$ -TM patients and 30 subjects as control groups). The age range of patients and control group was (15 - 40 years). This study was conducted in Teaching Baghdad Hospital - Baghdad and Al-Karama Hospital during the period from December 2022 to March 2023.

**Results:** The mean of serum Coenzyme Q10 was ( $3.55 \pm 1.62$  and  $2.02 \pm 0.88$  ng/ml) in  $\beta$ -TM patients and control group respectively. The results revealed that the mean of vitamin C was ( $6.85 \pm 3.0$  and  $12.23 \pm 2.99$  ng/ml) in  $\beta$ -TM and control group respectively. Also, the results shows that the mean of Vitamin E was ( $2.29 \pm 1.69$  and  $7.25 \pm 2.79$  ng/ml) in  $\beta$ -TM patients and control respectively. Also, the results revealed that mean of uric acid was ( $3.62 \pm 1.34$  and  $3.86 \pm 1.48$  mg/dl) in  $\beta$ -TM patients and control group respectively. The level of bilirubin was ( $1.99 \pm 1.25$  and  $0.65 \pm 0.6$  mg/dl) in patients and control group respectively. Also, the result shows there was a significant correlation between Coenzyme Q10 and Vitamin C ( $r=0.280$ ,  $p=0.030$ ) and non-significant correlation between Coenzyme Q10 and Vitamin E ( $r=0.084$ ,  $p=0.522$ ).

**Conclusion:** This study shows there were lower antioxidant capacities as demonstrated by the results in patients with  $\beta$ -TM patients in comparison with control group.

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## INTRODUCTION

Thalassemia is a hereditary hemoglobin condition characterized by decreased or nonexistent globin chain synthesis; clinical manifestations range from asymptomatic carriers to those who need lifelong transfusions and iron chelating treatment <sup>(1)</sup>.  $\beta$ -thalassemia is a heterogeneous group of hereditary hemoglobinopathies that are characterized by defects in the globin chain of hemoglobin. Thalassemia is classified as an autosomal recessive disorder, and approximately 1.5 percent of the population is made up of people who are carriers of the disease <sup>(2)</sup>. Reduced erythrocyte function causes chronic anemia, high morbidity, and a shorter life expectancy compared to the general population; those with severe anemia need regular red blood cell transfusions and iron chelation to prevent iron excess <sup>(3)</sup>. The most severe form, known as transfusion-dependent (TDT), is treated with potentially fatal blood transfusions (BT) for the rest of the patient's life <sup>(4)</sup>.

Vitamin C, or ascorbic acid, is a water-soluble vitamin that helps the body absorb iron, makes collagen, heals wounds, and keeps the immune system working properly <sup>(5)</sup>. In addition, it is a potent antioxidant, meaning it helps protect cells from harm; free radicals have been linked to a variety of diseases, including cancer, heart disease, and others. Vitamin C aids in both the absorption and storage of iron <sup>(6)</sup>. Beta-thalassemia major is an inherited disorder that prevents the body from making enough hemoglobin. Because hemoglobin is the protein in red blood cells responsible for transporting oxygen across the body, persons with beta thalassemia major suffer from severe anemia <sup>(7)</sup>. Antioxidants like vitamin C help prevent cell damage from oxygen radicals <sup>(8)</sup>. The beta-thalassemia gene due of their low hemoglobin levels and iron overload, major patients have a higher

risk of developing oxidative stress <sup>(9)</sup>. According to the findings of several research, taking vitamin C supplements might be beneficial for those who have beta-thalassemia major <sup>(10)</sup>. People with beta-thalassemia major whose vitamin C intake was increased on a daily basis had lower oxidative stress indicators and required fewer blood transfusions <sup>(11)</sup>. Supplementing with vitamin C significantly improved cardiac function and reduced oxidative stress in those with beta-thalassemia major <sup>(12)</sup>.

Uric acid, a waste product of an exogenous purine pool mostly sourced from animal proteins, is produced in the liver, intestines, and several other tissues such the kidneys, vascular endothelium, and muscles <sup>(13)</sup>. Purines are broken down in the body to generate uric acid, which is a typical waste product. Purines are found in a variety of foods, including liver, shellfish, and alcohol, and are used regularly by the human body. Internal production is possible as a consequence of DNA breakdown as well <sup>(14)</sup>. Uric acid is a powerful antioxidant produced by the body that protects cells from free radical damage <sup>(15)</sup>. Thalassemia major is a hereditary blood disorder that leads to anemia, fatigue, and other symptoms due to a defect in the generation of red blood cells <sup>(16)</sup>. It has long been known that uric acid protects against thalassemia major by decreasing inflammation and oxidative stress <sup>(17)</sup>. Uric acid levels are found to be lower in patients with thalassemia major compared to the general population, and studies show that increasing uric acid levels by supplementation may help reduce symptoms and improve quality of life <sup>(18)</sup>. Uric acid may be useful in treating thalassemia major, however further research is needed to determine the pros and cons of this medication, uric acid is being studied as a potential therapy for thalassemia major, but further study is needed <sup>(19)</sup>. Furthermore, uric acid supplements should

be avoided by some individuals since doing so increases the risk of kidney stones and other issues <sup>(20)</sup>. Uric acid supplementation, like the beginning of any therapy, requires a conversation with a medical practitioner <sup>(21)</sup>.

## MATERIALS AND METHODS

A case - control study was included 90 subjects aged from (15- 40 years) whom conducted Teaching Baghdad Hospital/Baghdad and AL-Karama Hospital, divided into two groups (60 patients with  $\beta$ -TM and 30 apparently healthy control, and applied the required test to measure important parameters that support our study. The study was implemented during the period from December 2022 to March 2023, and the study included patients previously diagnosed with beta-thalassemia major. According to the agreed protocol, a group of thalassemia patients who take blood every two weeks, especially patients before they were given blood, were taken.

### Data collection

Each person was given a questionnaire that has already been field-tested. After receiving extensive information regarding the trial, data collection, and research analysis, all patients consented to participate. After getting their permission in a one-on-one meeting, they were given a questionnaire to fill out. birthdates, body mass index, height, number of medications used, number of blood transfusions received, place of residence, risk factors, and illnesses.

### Sample Collection

The blood samples were drawn from the vein. Iodine was used to disinfect the venipuncture site, and five milliliters 5 ml of blood was drawn from each participant into a plane tube without anticoagulant; the blood was allowed to clot for 15-20 minutes; the sample was centrifuged for at least 10 minutes at 3000

rpm to separate the serum; the serum was then divided into small amounts and stored in Eppendorf tubes at - 20°C.

## RESULTS

### Demographic characteristics of patients and control group:

The current study included 60 patients with  $\beta$ -TM and 30 apparently healthy individuals serving as control group; the demographic characteristics of patient and control group was demonstrated in Table (1). There was no significant difference between patients and control groups. The minimum age in patients was 15 years, while in controls was 16, and the maximum age for both groups was 40.

**Table 1:** Descriptive statistics of age in study groups

Age (years)	Patients group	Control group
Mean	22.75	24.87
Std. Deviation	6.693	5.865
Minimum	15.00	16.00
Maximum	40.00	40.00
P value	0.114 <sup>NS</sup>	

NS: no statistical significance ( $p > 0.05$ ).

### Coenzyme Q10

Regarding serum CoQ10 level, the results in Table (2) showed that the CoQ10 level was higher among patient groups (3.55) when compared with the control group (2.02) with a highly statistically significant difference ( $p \leq 0.001$ ).

### Vitamin C

The present study revealed that the level of vitamin C in the patients was lower than that in the control group. The difference was statically highly significant ( $P < 0.001$ ), as shown in Table (3).

### Vitamin E

The results in Table (4) showed that there is a highly statistically significant difference in the mean vitamin E level in patients ( $2.29 \pm 1.62$  ng/dl) compared with a control group ( $7.25 \pm 0.88$  ng/dl).

**Table (2):** Descriptive statistics of CoQ10 in study groups

	Patients	control
Mean (ng/dl)	3.55	2.02
St. Deviation	1.62	0.88
P-Value	<0.001**	

**Table 3:** level of vitamin C in the study groups

Vitamin C level (ng/ml)	Patients group	Control group
Mean	6.85	12.23
Std. Deviation	3.0	2.99
P value	<0.001**	

Table (4): descriptive statistics of vitamin E in study groups.

	Patients	control
Mean (ng/dl)	2.29	7.25
St. Deviation	1.62	0.88
P-Value	<0.001**	

**Uric acid**

The present study revealed that the level of Uric acid in the patients was less than that in the control group. The difference was statically significant (P<0.435), as shown in Table (5).

Table 5: level of uric acid in the study groups.

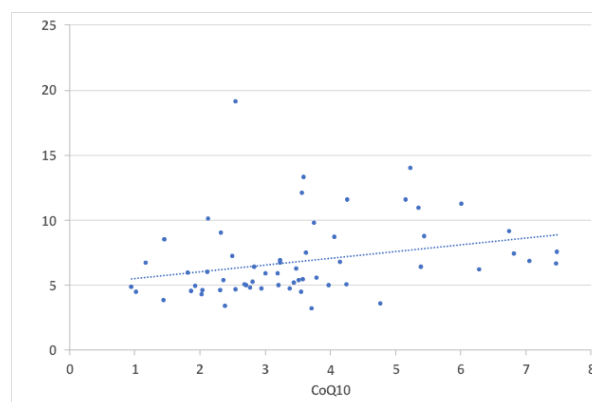
Uric acid level (mg/dL)	Patients group	Control group
Mean	3.62	3.86
Std. Deviation	1.34	1.48
P value	0.435	

**Correlation of CoQ10 with studied markers:**

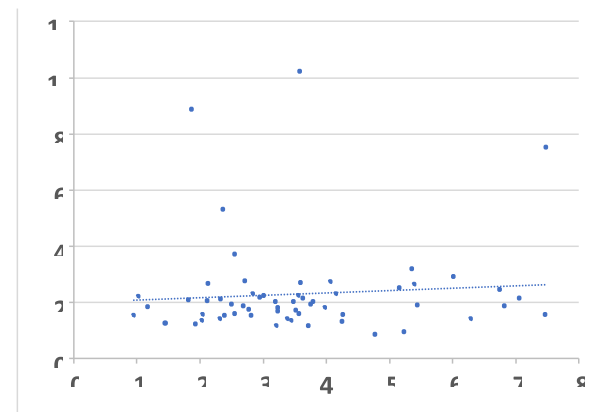
The results in Table (6) showed the correlation between serum CoQ10 level and other studied markers. The results showed that there is a positive correlation between CoQ10 level and vitamin C level (r=0.280, p=0.030) Figure (1). No significant correlation with vitamin E (r=0.084, p=0.522) Figure (2). No correlation with total bilirubin (r=0.142, p=0.277) Figure (3). No significant correlation with uric acid level (r=-0.066, p=0.615) Figure (4).

**Table 6:** Correlation of CoQ10 with other markers in patients.

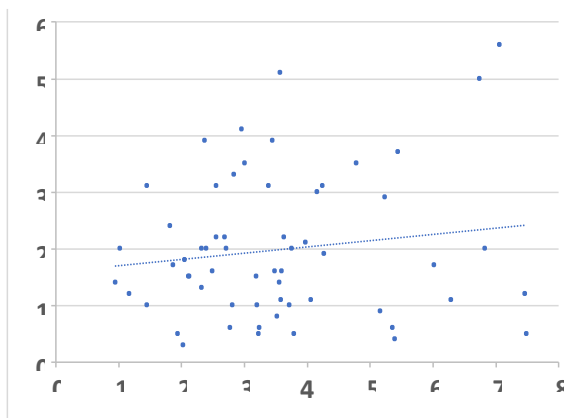
	Coenzyme Q10	
	r	P
Vitamin C	0.280	0.030
Vitamin E	0.084	0.522
Bilirubin	0.142	0.277
Uric acid	-0.066	0.615



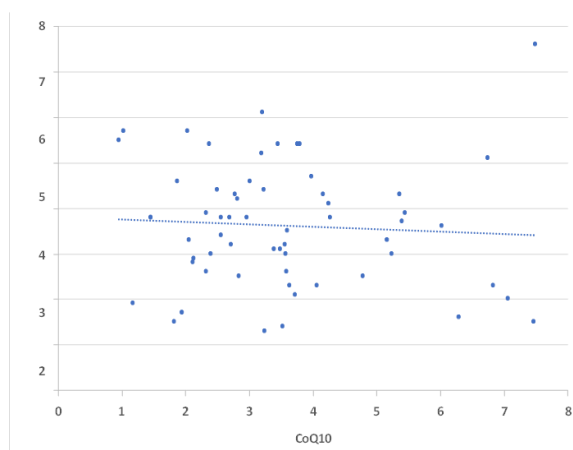
**Figure (1):** Scatter blot fitting between CoQ10 and Vitamin C



**Figure (2):** Scatter blot fitting between CoQ10 and Vitamin E



**Figure (3):** Scatter blot fitting between CoQ10 and total bilirubin



**Figure (4):** Scatter blot fitting between CoQ10 and uric acid

## DISCUSSION

The present study looked at how antioxidants affected patients with transfusion-dependent thalassemia major; findings revealed that patients were slightly younger than controls, but the difference was not statistically significant. The present study shows that the Coenzyme Q10 in patients with  $\beta$ -TM was significantly lower than of control group ( $p < 0.05$ ). these results go with Ruchaneekorn W. et al who reported that the levels of coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) are also very low in thalassemia<sup>(22)</sup>. as presented in Table (1). Our result agree with previous studies done by

Salman Z, Yılmaz T, Mehmetçik G, et al<sup>(23)</sup>, found that, demonstrated lower antioxidant capacity with decreased plasma CoQ10 concentration in  $\beta$ -TM patients. Oxidant and antioxidant status in children with  $\beta$ -TM and  $\beta$ -thal trait were investigated in several studies and most of them demonstrated decreased level of CoQ10<sup>(23)</sup>.

The present study revealed that the level of vitamin C in the patients with  $\beta$ -TM was significantly lower than that of control group ( $P < 0.001$ ), as presented in Table (2). agree with previous studies *Trapani S, Rubino C, et al.* found that, Sickle cell anemia and thalassemia patients who have had several blood transfusions may also have low vitamin C levels due to vitamin C being excreted in the urine<sup>(24)</sup>. Vitamin C, a vital antioxidant, may prevent cellular damage caused by free radicals<sup>(8)</sup>. Vitamin C is a water-soluble molecule that regenerates the radical form of alpha-tocopherol in addition to affecting the reduction of Fe<sup>+3</sup> to Fe<sup>+2</sup> and the absorption of iron from the stomach. These measures aid in controlling the redox balance<sup>(25)</sup>.

Also, our result agreement with *Dissayabutra et al.* Twenty TDT-affected children with low vitamin E and C levels for at least 6 months before the trial were given a 3-month course of vitamin C and vitamin E combination treatment. Glutathione, vitamin E, and vitamin C levels all increased by a substantial amount at the conclusion of the research, although malondialdehyde (MDA) and hemoglobin levels did not<sup>(26)</sup>.

The present study revealed that there was minimal significant difference in the level of uric acid in the patients compared with the control group ( $P < 0.435$ ), as presented in Table 4. This result agreement with previous studies *Sadeghi MV, Mirghorbani M, et al,* who found Hypercalciuria, impaired tubular

reabsorption of phosphorous TRP, hypomagnesemia, hypophosphatemia, and loss of renal magnesium were all symptoms of renal tubulopathy described by 14.6% of TM patients, along with renal hypouricemia, tubular proteinuria, and uric acid output <sup>(27)</sup>.

A previous study *Hassanein N, Thabet MAED, et al.* who found Tubular functioning and the probable hyperuricemia expected in thalassemic patients owing to the condition of continuous hemolysis and rapid erythrocyte turnover prompted additional examination of uric acid excretion among thalassemic patients <sup>(28)</sup>.

The present study revealed that the patient's bilirubin level was higher than that in the control group ( $P < 0.05$ ), as presented in Table 4.8. agree with *Shatha H. et al* <sup>(29)</sup>. Previous studies found that Due to the accelerated death of red blood cells in thalassemia patients, there is a high level of bilirubin in their blood. This is the main cause of hyperbilirubinemia, which has the side consequence of damaging other liver cells. who found that the bilirubin is another byproduct of erythrocyte hemolysis that is processed in the liver and retained in the gallbladder in the majority of beta thalassemia major patients. Elevated levels of total bilirubin also indicate a greater risk of bile duct constriction and gallstone development <sup>(29)</sup>.

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