

# Alterations in Antioxidants and Trace Element with Interleukin 6 Level in $\beta$ Thalassemia Major Patients

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## Abstract

$\beta$ -Thalassemia major is an inherited blood disorder caused by drop or total absence of beta globin chains. Patients with this blood disorder are repeatedly needed to blood transfusion to survive. There are many experimental and clinical evidence that suggested that the oxidative stress and free radical plays an important role in thalassemia. The aim of the present study was to investigate the level of antioxidant enzymes and trace element with interleukin 6 in  $\beta$  thalassemia major patients. The blood samples were obtained from 50 patients (30 with  $\beta$ -thalassemia major and 20 healthy controls). The serum levels of MDA, GSH, Vitamin (E, C), trace element (Fe, Zn, Cu, Se) and antioxidant enzymes (SOD, CAT, GPx) and interleukin 6 were analysed using conventional methods. The results showed that the level of MDA, Fe, Cu and interleukin 6 were significantly increased ( $P < 0.05$ ), whereas the activities of GSH, Vitamin (E, C), trace element (Zn, Se) and antioxidant enzymes (CAT, GPx) were decreased significantly ( $P < 0.05$ ) in  $\beta$ -thalassemia major patients compared with healthy control. This results suggested that the  $\beta$  thalassemia patients disease activity and progress could be investigated by determining the oxidative stress marker, trace element and interleukin 6 levels.

**Key words:** *Thalassemia major, Oxidative stress, Trace element, Interleukin 6, Vitamins.*

## Introduction

Alpha and beta thalassemia are known to be the most common types of thalassemia disease. The beta thalassemia major is found the very severe form that is required a repeated blood transfusions and treatment by desferrioxamine injections (1), Despite a such treatments could increase the patients' life span, however, it is related with a variety of complications, such as endocrine, skeletal metabolic, immunity, growth disorders. The trace elements and oxidative stress are detected as a consequence of high level of iron storage in the body and that might cause an oxidative stress. Though, some studies were suggested the damage caused by the endogenous free radical in thalassemia (2), the oxidative stress is the balance interruption among oxidants and reluctant in the body due to excessive production of both peroxides and free radicals caused cellular and tissue damage in the body, and this damage caused an oxidative stress and decrease the total antioxidant capacity (3). The production of the reactive oxygen species (ROS) that has the ability to react with all biological molecules such as lipids, proteins, carbohydrates and DNA, and exert a cytotoxic effects on

cellular components. Hence, increase ROS and impaired antioxidant defense are contributed for the initiation and progression of Beta thalassemia major disorder. The ROS activates is also diverse the damaging processes in cells, including oxidation of intracellular and surface components of the red blood cells in  $\beta$  thalassemia major patients (4). The antioxidants complex is Enzymatic antioxidants including catalase, superoxide dismutase and glutathione peroxidase, Superoxide dismutase and non-enzymatic antioxidants including glutathione vitamin A, C and E), and all are protect the key biological sites from oxidative damage and scavenge free radicals and other reactive oxygen species (ROS) (5). The trace elements play an important role in building of proteins, enzymes and complex carbohydrates to contribute in the biochemical reactions. Additionally, trace elements such as Zinc, Selenium, Magnesium, Manganese, and Copper are cofactors or structural components of antioxidant enzymes (6). In Beta thalassemia patients, the selenium element and glutathione peroxidase enzyme are observe an important protecting role of cell membranes from oxidative damage (7). Interleukin 6 (IL-6) it has a broad effect on immune and non-immune system related cells

and is often exhibit hormone-like characteristics that affect the homeostatic processes (8), and it has context-dependent pro- and anti-inflammatory properties. Several cytokines have been found at the chronic inflammatory sites, such as periodontitis autoimmune diseases, thyroiditis and arthritis. Also, there is an suggestion IL-6 is overproduced in thalassemia diseases (9).

### Material and Method

This study was conducted in Al-Mosul governorate, a 50 patients (30 with  $\beta$ -thalassemia and 20 were healthy controls) with age range (10-15) years old. The serums were collected by incubation the blood sample tubes in a water path at 37°C for 10 minutes, and then centrifuged at 13000xg using cooling centrifuge for 10 minutes. The supernatants were then collected and stored at -20 freezer (10).

serum Interleukin 6: The Interleukin 6 was analyzed by using ELISA technique (Eagle Biosciences, USA Kit) (11)

serum Malondialdehyde: The malondialdehyde levels was analyzed by method described by Yao-Yuan (12) .

serum Glutathione: The GSH concentration in serum was analyzed according to (13).

serum Vitamin C: The Ascorbic acid is oxidized by copper to form a dehydroascorbic acid and

diketogulonic acid. These products were treated with 2,4-dinitrophenylhydrazine (2,4-DNPH) to form the derivative bis-2,4- dinitrophenylhydrazone.(14).

serum Vitamin E: The Vit E was analyzed according to Emmerie-Engel reaction in which the tocopherols reduce ferric ion to ferrous ion, then it reacted with  $\alpha, \alpha'$ -dipyridyl give a red-orange color with absorbance at 520 nm.(15)

serum Superoxide dismutase activity: (SOD) activity was measured by colorimetric assay (16). We used commercially available colorimetric method (Randox Laboratories Ltd, UK).

serum Catalase activity: Catalase activity was estimated by the method of Aebi (17). Catalase can degrade hydrogen peroxide which can be measured directly by the decrease in the absorbance at 240 nm.

serum Glutathione peroxidase activity: Gpx activity was measured by the method (18).

serum trace elements: Zinc, Selenium and Copper were analyzed by atomic absorption spectrometry.

Statistical analysis: All data were reported as mean and  $\pm$  SEM. The statistical significance was assessed using Student's t-test. The P value less than 0.05 was accepted as the data were significantly different.

### Finding

**Table1: Descriptive data of studied for  $\beta$ -thalassemia patients and control individuals.**

Parameters	Control N=20	$\beta$ -thalassemia patients N=30
Age (Years)	11 $\pm$ 2.3	12 $\pm$ 4.4
Sex (Male/Female)	10/10	15/15
BMI (Kg/m <sup>2</sup> )	15.51 $\pm$ 1.23	16.1 $\pm$ 1.45
Duration of $\beta$ -thalassemia (years)	--	7.4 $\pm$ 2.4

Serum levels of MDA showed significant difference between  $\beta$ -thalassemia and control group ( $p < 0.05$ ). It was significantly increased in the  $\beta$ -thalassemia group when compared with control group. Per oxidative damage of lipids is indicated by the increase in serum MDA and significant decreased antioxidant defiance GSH & Vitamin when compared with control group ( $p < 0.05$ ) are shown in Table-2.

**Table 2: The levels of serum oxidative stress marker in  $\beta$ -thalassemia patients and control groups.**

Parameters	Control N=20	$\beta$ -thalassemia patients N=30
MDA ( $\mu$ mole/L)	2.66 $\pm$ 0.32	5.43 $\pm$ 1.2*
GSH ( $\mu$ mole/L)	12.95 $\pm$ 0.56	7.31 $\pm$ 1.1*
Vitamin C ( $\mu$ mole/L)	44 $\pm$ 2. 17	31 $\pm$ 2.2*
Vitamin E ( $\mu$ mole/L)	21.4 $\pm$ 1.5	13.2 $\pm$ 1.4*

Data are reported as mean  $\pm$  SD. \*P<0.05 compared to control (Student t-test).\* significant.

Table-3 showed that  $\beta$ -thalassemia patients significantly decrease significantly in glutathione peroxidase (GPx) and Catalase (CAT) level in serum compared with control groups (p<0.05). While superoxide dismutase (SOD) no significantly increase compared with control groups (p<0.05).

**Table 3: The levels of serum Activity enzymes and Interleukin 6 in  $\beta$ -thalassemia patients and control groups**

Parameters	Control N=20	$\beta$ -thalassemia patients N=30
CAT (U/ML)	3.1 $\pm$ 0.64	2.7 $\pm$ 0.7*
GPx(U/ML)	0.67 $\pm$ 0.11	0.55 $\pm$ 0.09*
SOD (U/ML)	6.82 $\pm$ 2.1	7.14 $\pm$ 1.7
Interleukin 6 (pg/ml)	1.99 $\pm$ 0.12	2.5 $\pm$ 0.13*

Data are reported as mean  $\pm$  SD. \*P<0.05 compared to control (Student t-test).\* significant.

The mean serum trace element (Zn , Cu , Se) levels are shown in  $\beta$ -thalassemia patients were significantly lower than control group, while (Fe) was significantly higher in serum than control group (Table-4).

**Table 4: The levels of serum trace element in  $\beta$ -thalassemia patients and control groups.**

Parameters	Control N=20	$\beta$ -thalassemia patients N=30
Zn ( $\mu$ g/dl)	107 $\pm$ 5.2	69 $\pm$ 1.4*
Cu ( $\mu$ g/dl)	107 $\pm$ 3.3	173 $\pm$ 2.4*
Se ( $\mu$ g/dl)	82 $\pm$ 2.2	69 $\pm$ 1.3*
Fe ( $\mu$ g/dl)	89 $\pm$ 4.3	163 $\pm$ 2.9*

Data are reported as mean  $\pm$  SD. \*P<0.05 compared to control (Student t-test).\* significant.

## Discussion

A significant increase serum iron proves patients have severe anemia due to ineffective erythropoiesis which is primary reason for iron overload and blood transfusion is secondary to it <sup>(19)</sup>. The results showed a significant lowering in Serum GSH levels in patients which was in agreement with many studies in normal and human diseases individuals <sup>(20)</sup>. Shekhar study showed a significant decrease of antioxidants, GSH in patients of low level of hemoglobin in red blood cells in beta thalassemia <sup>(21)</sup>. GSH is an essential antioxidant for recycling of vitamin E and C and is very powerful in helping the body fight against the free radicals <sup>(22)</sup>. GSH also participates in the cellular defense system against oxidative stress by scavenging free radicals and reactive oxygen intermediates. Decrease in GSH level in patients increases the sensitivity of cells to oxidative stresses <sup>(23)</sup>. Patne et al., found a significantly increasing of MDA in serum in  $\beta$ -thalassemia patients compared to healthy control <sup>(24)</sup>. MDA and acrolein ( $\text{CH}_2=\text{CHCHO}$ ), potentially toxic agents which spontaneously formed from aminoaldehydes, and induce oxidative stress in mammalian cells <sup>(25)</sup>. In  $\beta$ -thalassemia syndromes, decreased or impaired biosynthesis of beta-globin leads to accumulation of unpaired alpha-globin chains. Moreover, the iron overload in  $\beta$ -thalassemia patients generates oxygen-free radicals and peroxidative tissue injury and elevated MDA <sup>(26)</sup>. Vitamin C and E were significantly decreased in patients with  $\beta$ -thalassemia compared to healthy subjects. similar result was obtained by Dissayabutra et al. (2005), who showed a significantly lower in vitamin C and E levels in patients. This decrease may be due to multiple blood transfusion are at risk in iron overload and high oxidative. GPx This antioxidant enzyme belongs to a group of antioxidant selenoenzymes that protects the cells from damage by catalyzing the reduction of lipid hydro peroxides <sup>(28)</sup>. The reduction in GPx activity associated with enhanced oxidative stress in  $\beta$ -thalassemia may be related to increased  $\text{H}_2\text{O}_2$  levels <sup>(29)</sup>. The present study indicates a significant decreasing of CAT activity. The activity levels of CAT were significantly decreased in  $\beta$ -thalassemia males and females patients than in healthy subjects <sup>(30)</sup>. Therefore, a significant decrease in the levels of CAT indicated protection against oxidative stress <sup>(21)</sup>. Also no significant increase in SOD activity in patients compared with healthy subjects. In the present study, the activity of Erythrocyte SOD was no significantly ( $P>0.05$ ) increased as compared to controls. Erythrocyte SOD scavenges superoxide radicals to form

hydrogen peroxide and protects the cell membrane from its damage. Increased Erythrocyte SOD activity may be due to blood transfusion and increase in the proportion of younger erythrocytes, as a compensatory mechanism after increased oxidative stress <sup>(31)</sup>. A significant increase ( $p<0.001$ ) in serum iron was observed in beta thalassemia major when compared with controls. The patients have severe anemia due to ineffective erythropoiesis which is primary reason for iron overload and blood transfusion is secondary to it. Thus, increased iron may increase the potential of oxidative injury to erythrocytes and cell organelles <sup>(32)</sup>. Interleukin 6 (IL-6) is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. In humans, it is encoded by the IL6 gene <sup>(33)</sup>. In addition, osteoblasts secrete IL-6 to stimulate osteoclast formation. Smooth muscle cells in the tunica media of many blood vessels also produce IL-6 as a pro-inflammatory cytokine. IL-6's role as an anti-inflammatory myokine is mediated through its inhibitory effects on TNF-alpha and IL-1, and activation of IL-1ra and IL-10 <sup>(34)</sup>. IL-6 stimulates the inflammatory and auto-immune processes in many diseases such as  $\beta$ -thalassemia <sup>(35)</sup>. Diabetes <sup>(36)</sup> atherosclerosis <sup>(37)</sup> and multiple myeloma <sup>(37)</sup>. Thus, IL-6 concomitantly regulates proinflammatory and antiinflammatory activities and contributes to both the development and the resolution of the acute inflammatory response <sup>(35)</sup>. Tabatabaei et al reported that 84.8% of thalassemia major patients had zinc deficiency. They emphasized that the cause of zinc deficiency in these patients was due to insufficient zinc of dietary intake <sup>(37)</sup>. Yazdih et al showed that the serum concentration level of zinc in thalassemia patients ( $37\pm 1.9\text{mg/dl}$ ) was lower than in control group and there was significant difference statistically. They recommended zinc supplement for thalassemia patients <sup>(35)</sup>. They suggested that the etiology of zinc deficiency is malnutrition and inadequate zinc intake. They advise administration of zinc supplement <sup>(36)</sup>. In this study, the  $\beta$ -thalassemia major patients showed low levels of serum Se in comparison with the control group. These findings are comparable to the results reported by other studies <sup>(7)</sup>.

## Conclusion

This study indicates that in patients with  $\beta$ -thalassemia impairment of the antioxidant enzymes (GPx, CAT, SOD) along with essential trace elements (Se, Cu, Zn) minerals in order to reduce the extent of oxidative damage and the related complications in  $\beta$ -thalassemia major associated with elevated iron and

plasma levels of lipid peroxidation.

**Ethical Clearance:** taken from hospital and patients.

**Conflict of Interest:** Nil

**Source of Funding :** Nil

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