



## **Evaluation of serum antioxidant enzymes in $\beta$ - thalassemia major patients**

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**Abstract :** Seventy blood samples of clinically  $\beta$ -Thalassemia major patients were collected from thalassemic center pediatrics hospital in Babylon city / Iraq from individuals of both genders and different ages. Besides, blood samples were taken from 70 apparently healthy individuals (without Hemoglobinopathy disorders ) as a control group, during the period of October 2014 to February 2015. The results indicated that values for ferritin and urea were significantly increased in blood samples of thalassemic patients compared to controls ( $P < 0.05$ ). Levels of serum superoxide dismutase activity (SOD) were significantly increased in  $\beta$ -thalassemia patients compared to controls ( $967.43 \pm 115.6$  U/ml vs.  $170.7 \pm 40.2$  U/ml), while catalase (CAT) activity was significantly lower in thalassemic patients than controls ( $144.77 \pm 17.3$  U/ml vs.  $194.95 \pm 47.2$ )  $P$ -value  $< 0.05$ ). High levels of antioxidant enzyme SOD with decrease of antioxidant enzyme catalase, were associated with thalassemic patients compared to controls suggesting that assessment of hematological parameters and serum enzymes are valuable tools to predict thalassemia in Iraqi population.

**Keywords :**  $\beta$ - Thalassemia, Ferritin, Antioxidants.

### **Introduction**

Beta-thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the Far East as well as countries along the north coast of Africa and in South America<sup>1</sup>. Free radical production occurs continuously in all cells as part of normal cellular function. However, excess free radical production originating from endogenous or exogenous sources might play a role in many diseases<sup>2</sup>. Antioxidants are complex and diverse group of molecules that protect key biological sites from oxidative damage<sup>3</sup>. The major antioxidant systems include superoxide dismutase, catalase, glutathione peroxidase and recently more and more appreciated thioredoxin. While all of the above enzymes play important roles in the modulation of oxidative stress, the primary role in the metabolism of superoxide anion radical is exerted by superoxide dismutase<sup>4</sup>. Antioxidants are protective agents that have inactivated ROS and play an essential role in protection of the cells from oxidative damage<sup>5</sup>. They include several agents such as enzymes (glutathione peroxidase, superoxide dismutase, catalase), large molecules (ferritin, albumin), small molecules (uric acid, glutathione, bilirubin, ascorbic acid,  $\alpha$ -tocopherol, and vitamin E) and some essential trace elements such zinc,

**Anwar A. Abdulla /International Journal of ChemTech Research, 2018,11(07): 323-328**

DOI= <http://dx.doi.org/10.20902/IJCTR.2018.110738>

copper and selenium<sup>6</sup>. The activities of enzymatic antioxidants, catalase, glutathione peroxidase and glutathione reductase were found to be drastically reduced in untreated  $\beta$ -thalassemic patients when compared to the normal subjects<sup>7</sup>. The aim of this study is to evaluate the status of serum antioxidant enzyme levels in beta thalassemia patients.

## Material and Method

**Subjects and sample collection:** Seventy patients with  $\beta$ -Thalassemia major from thalassemic center pediatrics hospital in Babylon city, Iraq are taken, represent different ages and gender, and 70 samples healthy people (without hemoglobinopathy disorders) were randomly selected to compare with patients group in the study. Blood samples were collected by vein puncture in EDTA tubes from all patients and control groups.

### Chemistry Examination

- Ferritin.
- Glutamate Pyrovate Transaminase (GPT)
- Glutamic Oxaloacetic Transaminase (GOT).
- S. Urea

### Measuring of Antioxidant

#### Measurement of Catalase by ELISA

Human Catalase (CAT) ELISA (Cat No: E-EL-5408) kits were based on a sandwich-ELISA as the method, followed by the micro ELISA plate had been coated with antibody specific to CAT. Then biotenyated detection antibody specific for CAT and Avidin-Horsera dish peroxidase (HRP) conjugate were added to each micro plate well successively and incubated free components were washed away. The substrate solution was added to each well. Only those wells that contain catalase biotenyated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the colors turns yellow. The Optical density (OD) was spectrophotometrically at a wave length 450 nm. The concentration of the samples was calculated by the comparing O.D. of samples to the standard curve Figure (1).

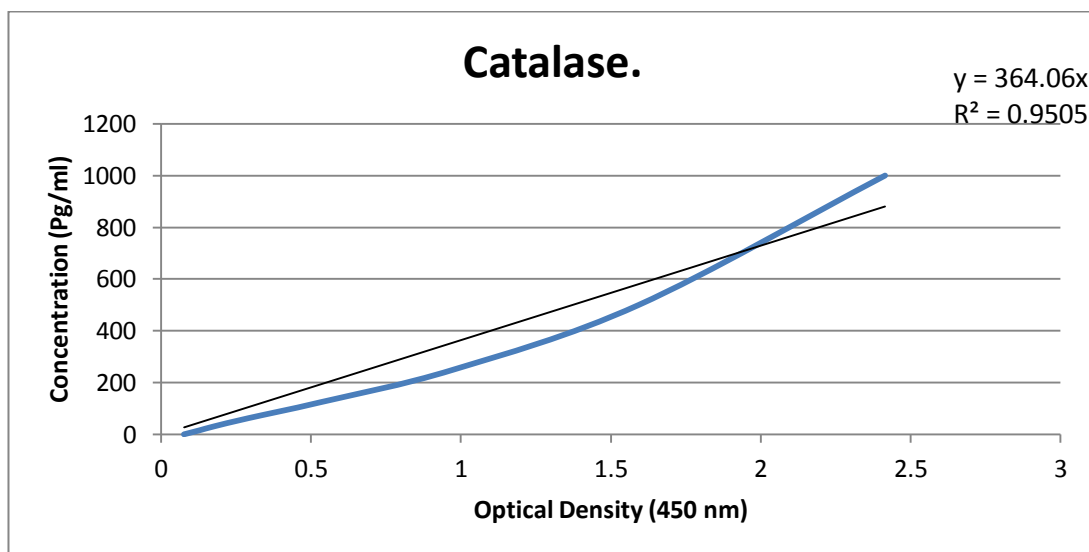


Figure (1): Standard curve for Catalase concentration.

### Measurement of Super Oxide Dismutase by ELISA

Human SOD ELISA (cat No: E-EL-H1113) kit were based on a sandwich-ELISA as the method, followed by the micro ELISA plate had been pre-coated with an antibody specific to SOD. During the reaction, Human SOD in the sample or standard competes with fixed amount of SODI on the solid phase supporter for sites on the biotinylated Detection Ab specific to Human SODI. Excess conjugate and unbound sample or standard are washed from the plate and Avidin-conjugate to Horseradish peroxidase (HRP) was added to each well. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the color change and then measured spectrophotometrically at wavelength 450nm. The concentrations of the samples were calculated by the comparing O.D. of samples to the standard curve Figure (2).

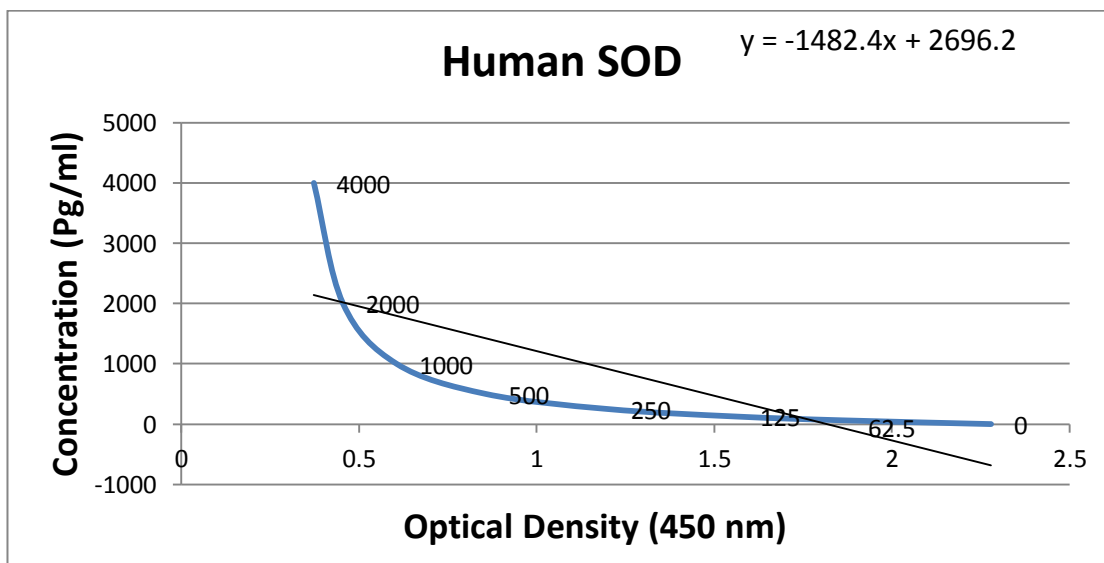


Figure (2): Standard curve for SOD concentration

### Biostatistical Analysis

The calculation of percentages, standard deviation estimation and t-test statistical analysis were applied for the analysis of results. Differences were considered significant at the probability of (P value <0.05) [5].

### Ethical Consideration

This study was conducted in strict adherence to the ethical guideline for conducting research on human subjects.

### Result and Discussion

The present study includes 70  $\beta$ -thalassemia patients who are currently being transfused and managed for the clinical symptoms and manifestations of the disease at the thalassemic center of thalassemia in Babylon pediatric hospital/ Iraq. Table (1) included (31) males and (39) females with ages range from (1-40 years).

Additionally, 70 samples were collected as control group included (42) males and (28) females with ages range from (3-40 years) from healthy people. The geographical distribution of the thalassemic patients reflects the heterogeneity of  $\beta$ -thalassemia within different regions of Babylon which may indicate that  $\beta$ -thalassemia can be occurred in all regions, races and ethnic groups.

**Table (1): Distribution of the samples according to the age categories**

Age group	Control (70)		Patients (70)	
	Number	%	Number	%
1-10 years	34	48.5	15	21.4
11-20 years	21	30	36	51.4
21- 30 years	13	4.2	16	21.4
31- 40 years	2	2.8	3	4.2
<b>Total</b>	<b>70</b>	<b>100</b>	<b>70</b>	<b>100</b>

The results of hematological examination (WBC, Hb, platelets) of 70  $\beta$ -thalassemia major patients were estimated as previously described <sup>8</sup>.

### Chemistry Examination

The examination of urea, GPT, GOT and Ferritin in serum of the patients as compared to the controls are presented in table (2).

**Table (2): Chemistry examination of  $\beta$ -thalassemia and compared with Control.**

Parameters	Control n=70	Patients n=70	p-value
	Mean $\pm$ SD	Mean $\pm$ SD	
Urea	3.3 $\pm$ 0.6*	4.8 $\pm$ 1.77	<0.001
GPT	9.5 $\pm$ 2.66*	12.7 $\pm$ 13.01	<0.001
GOT	7.6 $\pm$ 2.25*	12.7 $\pm$ 9.8	<0.001
Ferritin (ng/ml)	141.89 $\pm$ 83.8*	2854.89 $\pm$ 1923.8	<0.001

(Significantly different from control value (P < 0.05).

The results indicated that all parameters measured (urea, GPT, GOT), which markers for kidney and liver functions in the present study revealed significant differences between patients and controls. Walter *et al.* <sup>9</sup> reported a high level of serum urea and creatinine among patients of  $\beta$ -thalassemia. Severe anemia and chronic hypoxia shortened red cell lifespan and excess iron causes functional and physiological abnormalities in various organ systems, in  $\beta$ -thalassemia patients have a high prevalence of renal tubular abnormalities <sup>10</sup>. The blood transfusion is every (2-4) weeks to treated severe anemia which result 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 affection results solely from intravascular hemolysis, chronic transfusion or as a complication of iron chelation therapy <sup>11</sup>. Increased levels of GPT and GOT in  $\beta$ -thalassemia are due to iron overload, a very low hemoglobin level with very high (GPT, GOT) level along with splenomegaly is suggestive of poor prognosis in these patients <sup>12</sup>. While ferritin was significant differences between patients and controls.

The significant increase of serum iron and ferritin in Iraqi patients indicated an existing iron overload. The important elevation of ferritin was a major risk factor for myocardial infarction <sup>13</sup>. The repeated transfusion of blood causes iron overload in the  $\beta$ -thalassemia without chelation therapy that leads to many complications such splenomegaly and influence on liver function that leads to increase the (GPT,GOT) <sup>13</sup>.

### Antioxidant Enzyme

Superoxide dismutase activity (SOD) was significantly increased in  $\beta$ -thalassemia patients compared to the controls (967.43 $\pm$ 115.6 U/ml vs. 170.7 $\pm$ 40.2 U/ml), while catalase was significantly less than control (144.77 $\pm$ 17.3 U/ml vs. 194.95 $\pm$ 47.2), table (3).

**Table (3): The levels of SOD, Catalase in  $\beta$ -thalassemia compared with control**

Parameters	Control n=70	Patients n=70	p-value
	Mean $\pm$ SD	Mean $\pm$ SD	
Catalase	194.95 $\pm$ 47.2	144.77 $\pm$ 17.3	0.001
SOD	170.7 $\pm$ 40.2	967.43 $\pm$ 115.6	0.001

**Significantly (P value<0.05).**

The increased activity of SOD in  $\beta$ -thalassemia may be involved in scavenging the superoxideradical ( $O_2^-$ ), thereby producing more hydrogen peroxide in the erythrocytes<sup>14</sup>. In addition, the increase of intracellular antioxidant enzymes might be hypothesized to be a direct effect of increased intracellular iron on gene expression<sup>15</sup>. During the course of metabolism, superoxide anion was converted to  $H_2O_2$  by ubiquitous enzyme superoxide dismutase. Normally  $H_2O_2$  was converted to innocuous compounds by the action of catalase and peroxidase<sup>16</sup>.

But if free iron was available, it reacted with  $H_2O_2$  to form hydroxyl radicals which were extremely reactive species leading to depolymerisation of polysaccharide, DNA strand breakage, inactivation of functional proteins etc.<sup>17</sup>. The present results agree with other results that reported by Kassab-Chekir *et al.*<sup>15</sup>; Saud,<sup>18</sup> and they observed that iron, ferritin, SOD and GPX were increased in  $\beta$ -thalassemic patients. Increased red cell SOD values in thalassaemic patients have previously been explained as a reaction to, or compensation for, the increased production of superoxide radicals<sup>19</sup>. While the result of catalase was significantly decreased in  $\beta$ -thalassemia patients compared to controls, this result was agree with Hossian,<sup>19</sup> showed that SOD level is increased while possible explanation for lower red cell catalase level found in the more severe genotype of  $\beta$ -thalassemia is that the greater amount of hydrogen peroxide might cause direct toxic damage to catalase. The antioxidants increased in  $\beta$ -thalassaemia to protect the cells from damage of free radical that result from iron overload<sup>20</sup>.

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