Oxidative Stress and The Activities of Catalase , GST in Thalassemic Patients

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Abstract

The study concentrated on measuring the lipid peroxidation marker malon di aldehyde (MDA) in sera of thirty five patients with minor Thalassemia (Tm), thirty five with major Thalassemia (TM) and thirty five healthy control, their age ranged from (17-30) years.
Erythrocytes enzymes (Glutathione –S – transferase (GST)and Catalase) were assayed . The results showed a significant elevation in (MDA) in sera of both patient groups compared to control.
An increase in the activity of catalase and GST in erythrocytes of minor Thalassemic patients compared to control was found to be significant. Also, a high significant increase in the activity of GST for major Thalassemic patients compared to minor Thalassemic patients and control was found. On the other hand, the catalase activity for major Thalassemic patients was found in compatible with that of the control group.

Introduction

The Thalassemia are genetic disorders of hemoglobin in which there is a reduced rate of production of one or more of the globin chains e.g. alpha -chain or beta -chains (1).
Beta-thalassemia is the most familiar type (2),in which the beta – globin chain synthesis is impaired (3) .A deficiency of beta chain ,gamma chain synthesis continuos producing an increased proportion of HbF ,and delta chain production increases the amount of HbA2(4) .
The severity of a disease depends on the amount of HbA and Hb F , which present (5) .
In Iraq,thalassemia carrier rate for the country was found to be (4.8%) ,the rate was (5%) males , and (4.7%) females , with a higher rate in

urban areas (5%) than in rural ores (3.7%) .The highest carrier rate was in Basra (8.65%), while the lowest rate was found in Anbar (0%) , and in Baghdad (4.4%) (6) .

Thalassemic major (TM) patients are subjected to peroxidative tissue injury because of continuous blood transfusions (7). Thalassemia is a condition in which the erythrocytes are subjected to oxidative stress, such a condition can be related to toxicological events or to physiological causes(8).

severe oxidative stress occurs in the plasma of Thalassemic patients in comparison with the control(9).

Catalase is an unbiquitous heme protein that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen(10). There are diverse results recorded for catalase activity in erythrocytes of beta-Thalassemia (major and minor) as the activity of the enzyme react to the increase oxidant threat with augmented antioxidant enzyme activities (11).

GST enzyme is a biotransformation enzyme with many functions such as detoxification(12). It also catalyzes the reaction of a wide variety of electrophiles with glutathione in the GSH-dependent protection against lipid peroxidation (13)(14).

It was confirmed that the detoxifiying GSTs play an important role in the GSH-dependent protection against lipid peroxidation(15).The aim of this work is to evaluate the protective enzymes against lipid peroxidation catalase and GST in erythrocytes of thalassemia major patients and thalassiemia minor patients as pathological control compared to normal healthy subjects .

Experimental

Selection of subjects: One hundred five Thalassemia patients (male and female) were included in this study and classified as follows:

Group one of patients suffering from beta -Thalassemia major (TM), while the other group is beta -Thalassemia minor (Tm) . The blood samples were compared with the blood samples of thirty five normal healthy controls of comparable age and sex.

Blood sampling: Blood samples (8 ml) each was transferred into plain tube for serum separation .For measurmeant, MDA concentration Erythrocytes were washed three times with phosphate buffer saline where used for the determination of catalase and GST assay .Hemolysate preparation was done by placaing 0.1 ml RBC into 1.9 ml DW and vortex to complete hemolysis and centrifuged to remove the stroma .

Determination of hemoglobin :Hemoglobin was determined in whole blood and hemolysate by the method of cyanomethemoglobin using Drabkin`s reagent of commercially available kit (16).

Determination of lipid peroxidation levels (MDA): Lipid peroxidation was determined using Fong et.al .method (17).

Determination catalase activity in erythrocyte : Catalase activity can be determined using Acbi Hugo method (18).

Determination Glutathion –S- transferase (GST) activity in erythrocyte: Glutathion –S- transferase (GST) activity can be determined using Habig and Grancoise methods(19) (20) .

Statistical Analysis

Data presented were the means and standard deviations, student-t-test was used to compare the significance of the difference in the mean values of any two groups. ($P<0.05$) was considered statistically significant.

The overall predictive values for the results in all studied groups were performed according to program of office XP 2002.

Results and discussion

Malondialdehyde (MDA) is a compound derived from lipid peroxidation(21). MDA is still widely used in clinical chemistry laboratories to monitor oxidative stress (22).

Table (1) and fig.(1) show the results of MDA in both patients groups and control. A significant elevation was found in (MDA) for TM and Tm compared to control, also a significant higher levels was found in (MDA) of TM compared to Tm. There was a high significant elevation in (MDA) in all Thalassemia patients. Blood Platelet derived (MDA) may be a useful test of membrane damage in different patients with Thalassemia(23). Oxidative reaction in beta -Thalassemic patients are involved in premature cell removal and anemia(24).

The iron overload in beta -Thalassemic patients generates oxygen free

radicals and peroxidative tissue injury.

Free and total (MDA) levels were higher in the patients of Thalassemia major than in the other type. The results confirm the peroxidative status generateed by iron overload in Thalassemia patients and high light the rapid formation of marked amounts of free (MDA) despite the chelation therapy in Thalassemia major patients(25). The generation of Reactive Oxygen Species (ROS) is a steady-state cellular event in respiring cells.

Their production can be grossly amplified in response to a variety of pathophysiological conditions. The release of hemoglobin during hemolysis and the subsequent therapeutic transfusion in some cases lead to systemic iron overloading that further potentiates the generation of ROS(26) resulting in peroxidative damage to membrane lipids and proteins(27).

The results of catalase activity in erythrocyte of Tm and TM and control are presented in table (2) and fig. (2).

A significant high activity of catalase in RBC of Tm patients compared to TM and control, while the activity of catalase in TM patients where similar to that in the control group.

There was no significant differences in catalase among other antioxidant enzymes in beta -Thalassemic major(28).

Catalase was found to be greatly elevated in red blood cells of subject with beta -Thalassemia minor and similar to normal values in red blood cells subjects with beta -Thalassemia major, these findings allow to spenculate that red cells in beta -Thalassemia minor react to the increased oxidant threat with augumented antioxidant enzyme activities. The normal levels of antioxidant enzyme in beta -Thalassemia major seem to be due to the presence of normal cells owing to multiple transfusions(29).

The activities of catalase and other antioxidant enzymes were not significantly altered by dietary antioxidant in major Thalassemic patients(30).

On the other hand ,decreased activity of RBC catalase in beta -Thalassemic patients was reported which favors hydrogen peroxide e-mediated lipid peroxidation in those patients(31).

The activity of GST in RBC of all studied groups are shown in table (2) and fig. (2) a high significant increase in the activity of GST in TM patients compared to Tm and control was found, also a significant elevation in GST activity in minor Thalassemic patients

compared to control. In our study, GST activity in major Thalassemic was 5.7 times higher than in normal controls. A study conducted on (32) TM patients reports that GST levels were 5-10 times higher in patients with Thalassemia than in normal controls and age-matched leukemic patients, either reflecting extensive liver damage, elevated expression of the enzyme, or both in Thalassemic patients(33). beta - Thalassemia major is associated with varying degrees of liver damage, which may contribute to the elevated plasma GST levels in these patients(34).

Correlation relation: The correlation coefficient (r) test is used to describe the association between the different studied parameters $P<0.01$ was considered statistically significant.

Correlation relation between Catalase and GST: From figures (4), (5) and (6) shown a non significant positive correlation relation was between catalase and GST for control group with correlation coefficient r value (0.066), while a non significant negative correlation relation between catalase and GST for minor group with correlation coefficient r value (-0.248) but in major group show a significant positive correlation relation between catalase and GST with correlation coefficient r value (0.388).

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Table (1) MDA levels in sera of control , minor and major groups

Groups	No.	MDA (nmol/L) Mean ± SD	P
Control	35	12.9 ± 1.6	
Minor	35	30.1 ± 3.9	0.0001
Major	35	59.7 ± 8.4	0.0001
			0.0001 *

*Represent p value between minor and major groups

Table (2): Catalase & GST levels in erythrocyte of control , minor and major groups

Groups	No.	Catalase (U. gm –1.Hb) Mean ± SD	P	GST (U. gm –1.Hb) Mean ± SD	P
Control	35	1.5 ± 0.18		0.14 ± 0.03	
Minor	35	2.1 ± 0.19	0.0001	0.18 ± 0.01	0.0001
Major	35	1.5 ± 0.1	0.967	0.80 ± 0.09	0.0001
			0.0001 *		0.0001 *

*Represent p value between minor and major groups

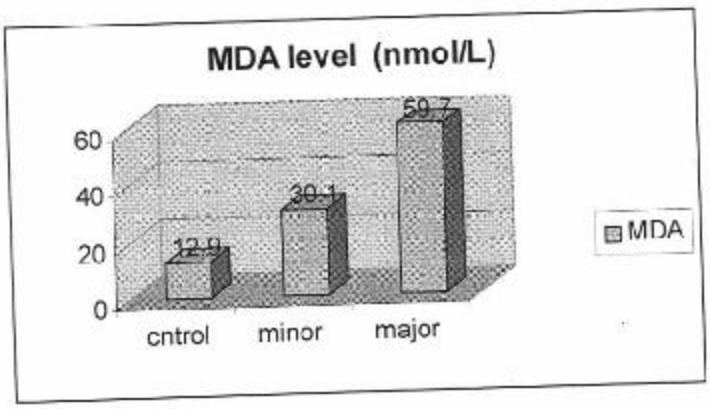

Fig. (1) MDA levels in sera of control, minor and major groups

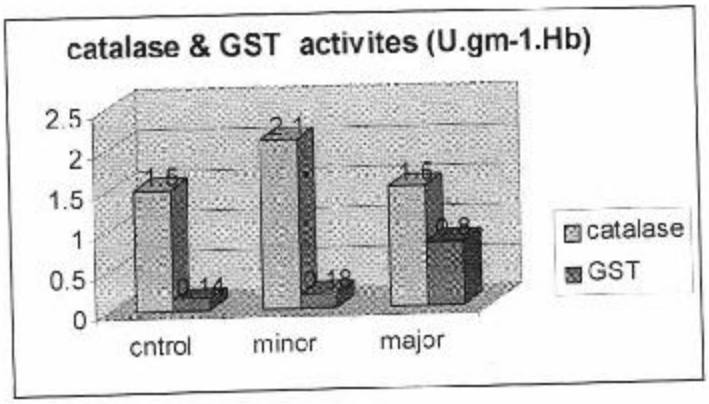

Fig. (2) Catalase & GST levels in erythrocyte of control, minor and major groups

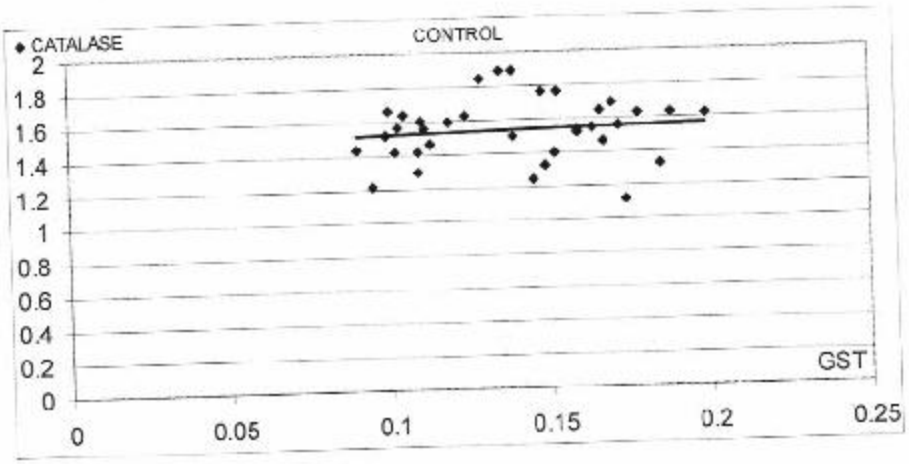

Fig. (3) Correlation relation between catalase and GST in control group

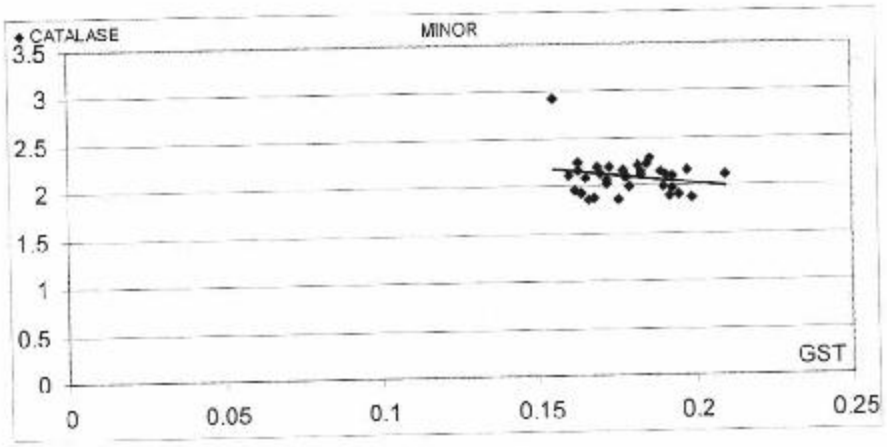

Fig. (4) Correlation relation between catalase and GST in Minor group

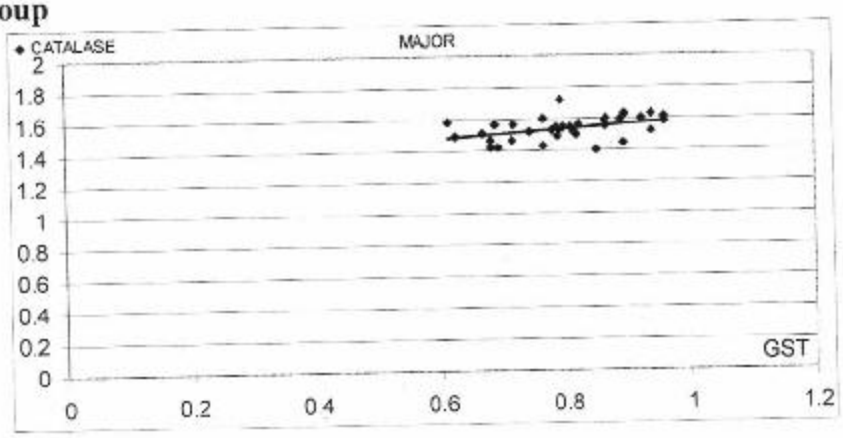

Fig. (5) Correlation relation between catalase and GST in Major group

الشد التاكسدي و فعالية أنزيمي الكتليز والكلوتا ثايون – س – ترانسفريز عند مرضى الثلاسيميا

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المستخلص

ركزت هذه الدراسة على قياس دليل الأكسدة الفوقية للدهون (المالون ثنائي الالديهايد)في مصل دم خمسة وثلاثين مريضا" يعانون من مرض فقر دم البحر الأبيض الأصغر وخمسة وثلاثين مريضا" يعانون من مرض فقر دم البحر الأبيض الأكبروخمسة وثلاثين شخصاً من الأصحاء كمجموعة سيطرة تتراوح أعمارهم بين (17- 30) عام . تم قياس فعالية انزيم الكتليز وانزيم الكلوتا ثايون – س – ترانسفريز في كريات الدم الحمر ، ظهر من النتائج ارتفاعا" معنويا" في (المالون ثنائي الالديهايد)في مصل دم مجموعتي المرضى مقارنة مع الأصحاء وكذلك وجدت زيادة معنوية في فعالية أنزيم الكتليز والكلوتا ثايون – س – ترانسفريز في كريات الدم الحمر لمرضى فقر دم البحر الأبيض المتوسط الأصغر مقارنة مع الأصحاء كذلك زيادة معنوية في فعالية والكلوتا ثايون – س – ترانسفريز لمرضى فقر دم البحر الأبيض المتوسط الأكبر مقارنة مع مرضى لمرضى فقر دم البحر الأبيض المتوسط الأصغر ومجموعة السيطرة بينما كانت فعالية أنزيم الكتليز لمجموعة فقر دم البحر الأبيض المتوسط الأكبر مقاربة لقيم مجموعة السيطرة .