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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(2):956-962. **OPEN ACCESS**

Evaluation of oxidative stress marker, antioxidant enzymes status and their impact on DNA damage in a group of Egyptian children with β-thalassemia

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Children with β-thalassemia are liable to serious effect of oxidative stress. DNA integrity and damage has a key role in the development of cancer and most human cancers are associated with DNA instability. Our study aimed to assess oxidative stress and antioxidant enzymes levels and to correlate them to DNA damage in children with β-thalassemia. This cross sectional case-control study conducted on 90 children who were categorized into three groups. Group I: 30 β-thalassemic patients with iron overload (serum ferritin > 1000 µg /dl); 10 of them received subcutaneous DFO (50 mg/kg daily for 5 days/week), 10 received oral deferiprone (75 mg /kg/day divided into 3 doses), 10 received subcutaneous DFO and oral deferiprone. Group II: 30 β-thalassemic patients who did not receive any iron chelation therapy, and their ferritin level is less than 1000µg /dl. Group III: 30 healthy age and sex matched children as a control group. There was significant increase in DNA fragmentation% in β-thalassemia when compared to controls. However, DNA fragmentation% significant decreased in patients received chelation than those who did not chelated yet. DNA fragmentation% was significantly lower in patients received DFP than those received DFO. In conclusion, SOD is significantly high in β-thalassemia patients and increase significantly after start of chelation therapy. Significant increase in total TGD, DNA in thalassemic patients compared to controls.

Keywords Thalassemia-, oxidative stress , chelation ,DNA fragmentation

INTRODUCTION

Thalassemia is one of the most common inherited single gene disorder caused by about 200 mutations in the beta globin genes results from the unbalanced production of hemoglobin chains in the red cell (1). Excess iron is toxic to the heart, liver, and endocrine system, in addition to the organ damage, excess iron can also lead to DNA damage by catalyzing the production of reactive oxygen species within the cell, leading to the induction of DNA damage and chromosomal aberrations (2). Lipid peroxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself, their formation occurs in enzymatic or non-enzymatic reactions involving activated chemical species known as "reactive oxygen species" (ROS) which are responsible for

toxic effects in the body via various tissue damages (3).

Oxidative stress in BTM patients activates various antioxidant enzyme systems to protect the body tissues from its damaging effects, a large number of antioxidant enzymes present in the body as superoxide dismutase, glutathione peroxidase (GPx), glutathione (GSH), glutathione S transferase, and catalase (4).

The aim of this work was:

To assess marker of oxidative stress (serum lipid peroxide) and antioxidant enzymes levels (superoxide dismutase, catalase and glutathione peroxidase) in β-thalassemia children.

To correlate between oxidant / antioxidant status and DNA damage in those patients.

MATERIALS AND METHODS

This study was a cross-sectional case-control study that was conducted on 90 children aged 3 to 18 years (40 male and 50 females). They were selected from the outpatient Pediatric clinic and the inpatients of pediatric department of Al-Zahraa University hospitals during the period from October 2014 to june 2016.

The studied children were divided into three groups:

Group I:

30 β-thalassemic patients with iron overload (serum ferritin level > 1000 µg /dl), and were on regular chelation therapy, and they classified to three subgroups:

Ia: 10 patients on regular chelation therapy with subcutaneous DFO (50 mg/kg daily for 5 days/week). they were 4 males and 6 females.

Ib: 10 patients on regular chelation therapy with oral deferiprone (75 mg /kg/day divided into 3 doses). They were 4 males and 6 females

Ic: 10 β-thalassemic patients on regular chelation therapy with subcutaneous DFO and oral deferiprone. They were 8 males and 2 females.

Group II:

30 β-thalassemic patients who did not start chelation therapy with iron chelators, and their ferritin level is less than 1000 (µg /dl).

Group III:

30 healthy age and sex matched children were taken as a control group.

All studied children were subjected to the following:

Full history taking, thorough clinical examination, and laboratory investigations

Routine investigations:

Complete blood count, reticulocyte count, random blood sugar, liver function tests e.g. ALT, AST, total and direct bilirubin, total protein and albumin, kidney function tests e.g. blood urea, serum creatinine.

Special laboratory investigation:

Oxidative stress marker (lipid peroxide) was detected by the Yagi methode.

Antioxidant enzymes (superoxide dismutase, glutathione peroxidase (GPx), catalase:

Superoxide dismutase was analyzed by the Minami and Yoshikawa methode.

Glutathione peroxidase activity was measured by the Paglia and Valentine.

Catalase was determined by method of Johansson and Borg.

DNA damage was estimated by: DNA fragmentation assay, electrophoretic pattern of nucleic acid, and Pro gel analysis technique.

Statistical Analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The quantitative data were presented as mean, standard deviations and ranges while qualitative data were presented as number and percentages.

RESULTS

There was statistically significant decrease in DNA fragmentation % in patients received chelation when compared to those without chelation therapy ($p>0.01$). Table (1) shows statistically significant decrease in DNA fragmentation % in patients with chelation when compared to patients without chelation therapy ($p>0.01$).

Table (2) shows highly statistically significant decrease in DNA fragmentation% in patients received mono chelation therapy (either deferiprone or dysferral) when compared to others on combined therapy ($P>0.01$).

Table (3) shows statistically significant decrease in mean serum GPx enzyme level and significant increase in mean serum level of MDA ($p >0.05$) among patients received chelation when compared to those without chelation therapy.

Table (4) shows statistically significant decrease in mean serum level of SOD in patients on monotherapy (either dysferral or deferiprone) when compared to patients on combined chelation therapy (p >0.01).

There was statistically significant increase in mean serum level of MDA in patients on single chelation therapy (either dysferral or deferoprone) when compared to patients with combined chelation.

Table (5) shows no statistically significant difference in DNA fragmentation % in splenectomized patients when compared to non splenectomized patients (p<0.05).

Table (1): Comparison between patients with chelation and patients without chelation therapy as regard DNA fragmentation percentage:

		Patients with Chelation	Patients without chelation	Independent t-test	
		No= 30	No= 30	T	P-value
DNA Fragmentation %	Mean ± SD	4.61 ± 4.66	7.66 ± 2.00	3.244	0.002
	Range	0.9 – 13	3.5 – 13		

p>0.01: significant

Table (2): Comparison between patients on different types of chelation therapy as regard DNA fragmentation %:

		Patients on dysferral	Patients on deferiprone	Patients on combined therapy	One Way ANVOA test	
					F	P-value
DNA Fragmentation %	Mean ± SD	1.44 ± 0.31	1.41 ± 0.14	10.99 ± 1.41	435.620	0.000
	Range	0.9 – 1.9	1.1 – 1.6	9 – 13		

P>0.01: highly significant.

Table (3): Comparison between patients with chelation and patients without chelation as regard antioxidants enzymes and oxidative stress marker (MDA):

		Patients with Chelation	Patients without chelation	Independent t-test	
		No= 30	No= 30	t	P-value
SOD u/ml	Mean ± SD	20.46 ± 19.85	48.59 ± 7.28	7.163	0.000
	Range	4.3 – 56	38 – 65		
GPx u/ml	Mean ± SD	263.37 ± 12.82	287.59 ± 33.59	3.682	0.001
	Range	226 – 289	227 – 359		
CAT	Mean ± SD	35.16 ± 21.65	6.44 ± 1.02	-7.134	0.000
	Range	4.8 – 59	4.3 – 8.3		
MDA nmol/ml	Mean ± SD	7.31 ± 4.25	1.24 ± 0.35	-7.518	0.000
	Range	1.3 – 14.4	0.4 – 1.9		

P>0.01: highly significant.
P>0.05: significant

Table (4): Comparison between patients on different types of chelation therapy as regard serum antioxidants enzymes and serum MDA level:

		Patients on dysferral	Patients on deferiprone	Patients on combined therapy	One Way ANVOA test	
					F	P-value
SOD u/ml	Mean ± SD	5.89 ± 1.00	7.03 ± 0.75	47.00 ± 5.44	501.154	0.000
	Range	4.3 – 7	6.1 – 8.3	38 – 56		
GPx u/ml	Mean ± SD	263.50 ± 10.19	264.00 ± 7.92	262.60 ± 19.03	0.029	0.972
	Range	255 – 289	248 – 276	226 – 289		
CAT mu/l	Mean ± SD	48.90 ± 3.76	49.90 ± 6.85	5.68 ± 0.48	318.865	0.000
	Range	44 – 56	40 – 59	4.8 – 6.4		
MDA nmoe/ml	Mean ± SD	10.57 ± 1.86	9.73 ± 0.81	1.63 ± 0.18	175.660	0.000
	Range	8.8 – 14.4	8.3 – 11.4	1.3 – 1.9		

p>0.01: highly significant. P<0.05: no significant.

Table (5): Comparison between splenctomized and non splenctomized patients as regard DNA fragmentation %:

		Splenectomized	Non Splenectomized	Independent t-test	
				t	P-value
DNA Fragmentation %	Mean ± SD	6.02 ± 3.88	6.26 ± 4.00	0.232	0.818
	Range	0.9 – 12.1	1.1 – 13		

P<0.05: no significant.

DISCUSSION

The DNA repair system is a cellular defense mechanism responding to DNA damage caused in large part by oxidative stress, the DNA damaging agents are either external or internal such as metabolic pathways that produce reactive oxygen species (5).

Our results showed that the mean and SD of DNA fragmentation percentage was significantly higher in BTM patients (6.11 ± 3.89) % when compared to controls (0.84 ± 0.22) %.

This finding is consistent with the study of El-Gendy et al., (6), who studied the total genomic damage (TGD) of DNA by conventional gel electrophoresis among 70 studied BTM patients and found that there was highly significant increase in TGD of DNA in thalassemic patients when compared to control groups.

Our results also agreed with El-Rashidy et al., (7) the study included 90 subjects to evaluate the DNA damage in ßTM and found that they were suffering of double strand breaks of DNA of their peripheral leukocytes (9.46±10.37) % when compared to control (1.49±2.64) %.

A possible explanation of the background TGD of the DNA in the thalassemia patients, may reside in the higher sensitivity of their leukocytes to the effect of genotoxic drugs or mutagenic food additives that may be consumed by them (8).

Despite absence of high iron overload in control group, there was TGD of DNA which raise the attention that, there may be other factors resides beyond this damage.

Also, there was statistically significant decrease as regard DNA frag % between patients with chelation therapy (4.61 ± 4.66) % when compared to patients without chelation therapy (7.66 ± 2.00) %, .This result compatible with El-Rashidy et al., (7) who found statistically significant decrease in TGD in B thalassemic chelated patients (25.83±22.48) % when compared to non-chelated patients (35.66±23.89) %, these results indicate that thalassemia patients with high iron overload had significant increase in the TGD of DNA.

And there was highly statistically significant increase in DNA fragmentation % between BTM patients on desferrioxamine therapy (1.44 ± 0.31) % when compared with others on deferiprone therapy (1.41 ± 0.14) %.

Deferiprone had significant protective effect against DNA damage compared to DFO, these results are consistent with the findings of Marshal et al., (9), who studied the effect of DFP and DFO on DNA damage in a group of Indian thalassemia, where he revealed that chromosomal aberrations were less frequent in patients treated with deferiprone than patients treated with DFO, despite the difference did not reach statistical significance.

This result showed that the iron chelation with DFO may lead to improvement of the total DNA damage rather than worsen it, which agreed with El-Rashidy et al., (7), who found a decrease in the frequencies of TGD of DNA were observed following regular therapy with DFO.

Superoxide dismutase is the main ROS, which react with nitric oxide radical and forms peroxynitrite, causing oxidative stress and cellular damage, SOD is the essential antioxidant that decreases the formation of ROS and oxidative stress; thus, protecting the cells from damage, erythrocyte SOD protects the erythrocyte from being damaged during oxidative stress, SOD activity in patients with BTM is decreased, resulting in pronounced inhibition of the blood antioxidant (10).

As regard mean level of SOD, our results showed that the mean and SD of SOD enzyme is highly statistically significant increase in thalassemic patients (34.52 ± 20.52) u/ml when compared to controls (7.44 ± 0.94) u/ml.

This result agreed with Simsek et al., (10), who studied the oxidant and antioxidant status among 11 regularly transfused BTM patients and their status were compared with 10 sex and age-matched healthy subjects and found that Erythrocyte superoxide dismutase (ESOD), and plasma malondialdehyde were found to be higher in thalassemia major patients than controls.

Increased levels of antioxidants, including

SOD, may be due to blood transfusion and increase in the proportion of younger erythrocytes, also a compensatory mechanism after increased oxidative stress including an acute inflammatory phase, a state of trauma, and upon exposure to increased levels of pro-oxidants (11).

It could be explained with the inability of the antioxidant system to compensate excessive originators, free radicals could not be offset by the system, which may have caused the degradation of proteins which in turn decreased the levels and activity of antioxidant enzymes, this is supported by other publications, which state that chronic stress in diabetes mellitus, metabolic syndrome, chronic liver disease, SLE, and rheumatoid arthritis affect the decrease in antioxidant enzyme capacity (12).

Also there was highly statistically significant decrease in SOD between BTM patients on chelation therapy (20.46 ± 19.85) u/ml, when compared to patients without chelation therapy (48.59 ± 7.28) u/ml.

Elalfy et al., (13), found that mean SOD was significantly decreased ($p<0.001$) during the study period compared to baseline.

Also there was decrease in mean serum SOD level in group treated with DFO (5.89 ± 1.00) u/ml than others treated with DFP (7.03 ± 0.75) u/ml.

This finding agreed with (Ozturk et al.,) who studied the plasma levels of peroxiredoxin 2 (Prx2), thioredoxin 1 (Trx1), and the activity of thioredoxin reductase (TrxR), in TM patients living in the Antalya region, Turkey, the patients were divided into two groups, according to chelators, the deferoxamine group (DFO, n _ 20), and the deferiprone group (DFP, n _ 20), to compare the possible effect of chelators on anti-oxidative and oxidative stress parameters and their results revealed reduction in the level of SOD in DFO group (3.58 ± 0.25) u/ml than other DFP group (3.70 ± 0.22) u/ml.

As regard mean red cell GPX level, our study demonstrated highly statistically significant reduction in GPX in BTM patients (275.27 ± 27.86) u/ml when compared to control group (312.67 ± 45.19) u/ml,

And also our results matched with Mahdi who showed that there was statistically significant decrease of GPX in thalassemia children (5.35 ± 1.2) U/L when compared to controls (15.45 ± 5.87) U/L.

Our study showed that there was statistically significant decrease in mean serum GPx level among patients with chelation therapy (263.37 ± 12.82) u/ml when compared to patients without chelation therapy (287.59 ± 33.59)u/ml.

This result matched with Attia et al., (16), who detected the effects of chelation therapy on antioxidant status and liver function in homozygous βT patients and found GPx activity in RBCs was significantly reduced in group with chelation therapy (23.5±8.24) u/ml than other non-chelated group (43.89±9.5)u/ml, ($p < 0.05$).

Our results showed decreased GPx in group receiving DFO (263.50 ± 10.19) u/ml than others receiving DFP (264.00 ± 7.92) u/ml, this agreed with Ozturk et al., (14) study, this study was compared the possible effect of chelators on anti-oxidative and oxidative stress parameters among BTM group on regular chelation therapy and their results revealed the activity of GPx is in DFO group (70.03 ± 6.48) u/ml lower than DFP group (78.32 ± 6.60)u/ml.

As regard the mean and SD of CAT enzyme level, our study revealed highly statistically significant decrease in BTM patients (21.05 ± 21.08) MU/L and control group (50.60 ± 6.85) MU/L.

And also agreed with Mahdi (15), his study was to investigate the relationship between oxidative stress by measuring the role of antioxidant enzymes including SOD, CAT, GSH, GPX in thalassemia blood samples, from results obtained, it was clear that there was a significant decrease in catalase activity of βTM patients (44.88 ± 19.87) MU /L when compared to control group (120.29 ± 25.2) MU /L.

While (Addour et al.,) showed no statistically significant change in CAT among BTM patients group when compared to healthy one, which possibly due to the presence of normal red cells owing to multiple blood transfusions.

Also our findings detected that there was highly statistically significant increase in mean serum catalase levels in patients with chelation therapy (35.16 ± 21.65) MU/L when compared to patients without chelation therapy (6.44 ± 1.02) MU/L that possibly explained as a compensatory mechanism for the reduced glutathione peroxidase activity in red blood cells (18).

In our study, we detected increased level of mean and SD of catalase enzyme in group received deferiprone (49.90 ± 6.85) MU/L more than other received desferoxamine (48.90 ± 3.76) MU/L.

This matched with (Ozturk et al.,) their study was to compare the possible effect of chelators on anti-oxidative and oxidative stress parameters among BTM patients group on regular chelation therapy and their results detected that higher CAT

activity was observed in the DFP group (16.30 ± 0.46) MU/L than DFO (15.12 ± 0.45) MU/L group.

As regard mean and SD of MDA, Our study revealed that, there was statistically significant increase in mean serum level of MDA between B-thalassemia cases (6.51 ± 1.25) nmol/ml, and control (4.38 ± 4.32) nmol/ml.

This result agreed with (Choudhary et al.,) who study, the relationship between oxidative stress and serum antioxidant enzymes level in thalassemia, the study was done on 50 cases and 50 control subjects from 3 to 14 years, by using fresh samples, MDA, GSH, SOD and CAT were tested and found that MDA level was higher in the thalassemia group (17.16±1.79) nmol/mL than control group (8.99±1.33) nmol/mL.

Our results showed that there was highly statistically significant increase in mean and SD of MDA level in patients with chelation therapy (7.31 ± 4.25) nmol/ml when compared to patients without chelation therapy (1.24 ± 0.35) nmol/ml.

This result agreed with(Laksmiwati et al.,) who found that Peroxidation products of (poly)-unsaturated fatty acids in membrane phospholipids such as thiobarbiturate-reactive substances (e.g. MDA) are 5.0 nmol/mL in non-chelated thalassemia patients and 8.7 nmol/mL in chelated patients.

(Das et al.,) revealed the treatment with chelation therapy for a period of six months remarkably reduced the level of lipid peroxidation in erythrocyte membranes

Also our study showed that mean and SD of MDA level in patients received desferoxamine (10.57 ± 1.86) nmol/ml was higher than those received deferiprone (9.73 ± 0.81) nmol/ml.

This result compatible with (Ozturk et al.,) this study was to compare the possible effects of chelators on anti-oxidative and oxidative stress parameters among BTM group on chelation therapy and their results detected that the levels of MDA in the DFP group (25.31 ± 1.15) nmol/ml were lower than those in the DFO group (28.50± 1.07) nmol/ml (p >0.05), and defined the highest measure of MDA levels in the DFO group may also show elevated oxidative stress in this group.

The significant increase in MDA and the decrease in GPx indicated increased oxidative stress, possibly due to the iron overload in the TM patients, iron chelators did not seem responsible for the differences between anti-oxidative and oxidative stress parameters, although there were significant differences among the groups, these differences were most likely because of different ferritin levels in the groups (22).

CONCLUSION

Beta thalassemia patients tend to have low antioxidant enzymes activity (GPX and CAT) and higher oxidative stress (MDA).

Patients with combined chelation therapy showed significant decrease of GPXZ and CAT in comparison to patients with single chelation therapy.

Significant increase in total TGD, DNA in thalassemic patients which decreases significantly in patients received chelation.

There was an inverse relation between DNA frag % and frequency of blood transfusion.

CAT, DNA frag %, MDA and SOD were able to achieve a relatively high sensitivity of 100% and specificity 100% in patients without chelation therapy and control.

CONFLICT OF INTEREST

The authors declared that the present study was Performed in absence of any conflict of interest

ACKNOWLEGEMENT

All appreciation to participants' children and their parents for their time and help

AUTHOR CONTRIBUTIONS

AGM performed a clinical assessment to the study subjects. HAS and AGM, MAE wrote the manuscript. AMG, SNR, performed data analysis and reviewed the manuscript. AGM and SSK performed the laboratory work. All authors read and approved the final version

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