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Lipid peroxidation in beta-thalassaemia

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Abstract. In this study, 82 patients with β-thalassaemia major, 116 patients with β-thalassaemia minor and 142 apparently healthy control subjects were included. For all three groups, the following parameters were estimated: haemoglobin and blood indices, serum iron, total iron binding capacity, transferrin saturation, serum ferritin, zinc and copper. Malondialdehyde (MDA) as a marker of lipid peroxidation was also determined. The results of haematological investigations revealed that the mean haemoglobin level was (6.6±1.6gm/dl) for patients with β-thalassaemia major while for subjects with β-thalassaemia minor and controls were (11.1±1.8 gm/dl and 12.4±1.5 gm/dl respectively). Other blood indices (PCV, RBCs count, MCV, MCH and MCHC) were severely altered in patients and it was milder in carriers compared with control subjects. Lipid peroxidation (as measured by estimating serum malondialdehyde) was significantly elevated in the patients and carriers (1.14±0.31 μmol/l and 0.86±0.2 μmol/l respectively) as compared with control (0.67±0.19 μmol/l), P<0.001. Among thalassaemia major patients there was a significant positive correlation between MDA concentration and transferrin saturation (r=0.42, P<0.01). While, a significant positive correlation between MDA and serum copper in both thalassaemia minor and major patients (r=0.4, P<0.01; r=0.52, P<0.01, respectively) was observed. In addition to that a significant negative correlation between serum MDA and serum zinc levels was present in patients with β-thalassaemia major only (r=-0.55, P<0.01). The study has illustrated also that splenectomy in patients with β-thalassaemia major affects all the estimated parameters but this was only statistically significant in serum MDA level (P<0.001) as compared to the controls. In conclusion, our study demonstrated an increased level of serum MDA in both thalassaemia minor and major patients, thus indicating increased lipid membrane peroxidation.

Key words: β-thalassemia major • lipid peroxidation • MDA • children Abstract

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INTRODUCTION

Red cells are at increased risk of oxidative degeneration process that leads to aging and cellular breakdown due to its exposure to high concentration of oxygen and its inability to replace damaged components by resynthesis[1]. There is a continued spontaneous source of activated oxygen within the red cell, when the haeme iron loses an electron resulting in the formation of ferric methaemoglobin[1].

The conformational changes as in the unstable haemoglobin and isolated haemoglobin chain cause increased methaemoglobin formation and superoxide release in thalassaemia[2,3]. An additional important source of activated oxygen is the interaction of heavy metals ion with

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oxyhaemoglobin. Copper ions at high levels; greatly stimulate haemoglobin auto-oxidation, which involve superoxide production[4,5]. Iron produces a similar effect to copper, and non-transferrin bound iron is a potentially toxic compound, which is increased in the serum of patients with iron overload[6].

The peripheral red cells of patients with β-thalassaemia major demonstrate a variety of morphological, biochemical and metabolic changes, which specifically contribute to the extent and severity of lipid peroxidation and haemolysis[7]. These abnormalities include; the composition of membrane lipid that are nearly two folds increased compared to normal subjects, thus increased lipid peroxidation due to oxidative stress is common in β-thalassaemia[8-10]. The free α-chains in β-thalassaemia increase auto-oxidation rate by about two times faster man in normal Hb A[11]. In addition to that iron can serve as a potent catalyst of lipid peroxidation[6,10,12,13] as well as the presence of catalytic amount of copper[9,11] and at last, there is some evidence that the presence of hypochromia may facilitate oxidation of the red cell membrane by reducing the amount of haemoglobin available for this buffering protection[7,14].

The aim of the study was to find out the effect of iron status, serum zinc and copper levels on lipid peroxidation calculated by malondialehyde production in subjects with β-thalassaemia minor and major and to study the correlation and interaction between different parameters and lipid peroxidation.

SUBJECTS AND METHODS

Subjects

This study was conducted, from July 2000 to July 2001 on 82 patients with β-thalassaemia major, aged 1 to 32 years (48 males and 34 females), who attended the Thalassemia Center in Basrah Maternity and Children Hospital, and a second group of 116 patients with β-thalassaemia minor; mostly the thalassaemia major patients relatives aged 1 to 60 years (47 males and 69 females). In addition to patients, 142 age-matched healthy subjects (with normal haemoglobin pattern), aged 1 to 61 years (69 males and 73 females) had served as a control group.

Each of the patients and control groups were categorized into four main subgroups according to their ages, group A (1-6 years), group B (7-12 years), group C (13-18 years) and group D (included subjects aged more than 18 years).

From patients with β-thalassaemia major, the following data were collected; age, sex, age of diagnosis of the disease, complaints, frequency of blood transfusion, data of last transfusion, chelating therapy (dose, route and duration), splenectomy, weight and height. While for those with β-thalassaemia minor and controls only age, sex, weight and height were recorded. Body mass index (BMI) was measured as weight (in kilograms) divided by the square of the height (in meters).

Methods

The importance of the procedure was explained to subjects participated in the study and/or their parents. Fresh venous blood (6-8 ml) was collected from patients and control subjects. About 2 ml was added to EDTA tubes; immediately tested for haemoglobin variant and haematological parameters. The reminder was transferred to plain tubes (without anticoagulant), centrifuged and the sera were obtained for the biochemical investigations.

For the thalassaemia major subjects, the samples were collected just prior the blood transfusion. Haemoglobin, PCV, red cell counts, MCV, MCH and MCHC were estimated by using automated Coulter Counter MS9. Haemoglobin typing was performed quantitatively by an automated ion exchange HPLC system using β-thalassaemia short programme on the Bio-Rad VARIENT instrument (Bio - Rad Laboratories, Belgium). β-thalassaemia trait or minor was identified by the characteristic elevation of HbA_2 (more than 3.8%).

Serum iron and total iron binding capacity were tested within 24 hours by spectrophotometric method[15]; transferrin saturation (%) was calculated mathematically. Malondialdehyde (MDA) has been identified as the product of lipid peroxidation that reacts the thiobarbituric acid to give a red species absorbing at 535 nm[16], was also tested with 24 hours. The reminder sera were stored in a deep-freeze (-20 ^{o}C), for the later estimation of serum Zn, Cu, by using direct method with acetylene and air flame atomic absorbance spectrophotometer (FAAS) after simple dilution[17]. Whereas serum ferritin estimation was carried out by radioimmunoassay technique as described by CIS bio leaflet enclosed with the kit

Statistical Analysis

The results were expressed as mean ±SD. The data were analyzed statistically by one-way analysis of variance (ANOVA). While the correlation between the data were tested statistically using simple linear regression, employing SPSS computer program. $P<0.05$ was considered to be the lowest limit of significance.

RESULTS

The haematological findings in both thalassaemia major and minor as well as their control group are shown in Table 1. As illustrated, the haemoglobin level (Hb), packed cell volume, mean cell volume (MCV) and mean cell haemoglobin (MCH) were all significantly decreased in both β-thalassaemia major and minor individuals (P<0.001). Red blood cells count was significantly higher in β-thalassaemia minor individuals as compared with control subjects (p<0.001), although it was significantly lower in β-thalassaemia major patients as compared with healthy control.

The basic biochemical characteristics, which were investigated in this study for all groups are presented in Table 2. The serum levels of iron, ferritin, copper, Cu/Zn ratio, malondialdehyde (MDA) and transferrin saturation were all increased in both β-thalassaemia major and minor as compared with controls, and these increases were statistically significant compared with each other and with control group (p<0.001). While there was a significant decrease in serum zinc levels in thalassaemic (major and minor) patients as compared with the controls, (p<0.001).

The degree of lipid peroxidation in different age groups was evaluated by the estimation of serum MDA and the results are presented in Table 3. The result reveals that serum MDA levels were significantly increased in patients with β-thalassaemia major in all age groups as compared with age matched apparently healthy control (p<0.01). While in β-thalassaemia minor it was significantly higher in patients over 18 years old (p<0.001) as compared with age-matched control. In regards to the difference between β-thalassaemia major and minor, serum MDA levels were significantly higher in all age groups in β-thalassaemia major as compared with β-thalassaemia minor except for the patients under 6 years (p>0.05).

In patients with β-thalassaemia major, 24 patients were splenectomized and 58 were not, serum iron, transferrin saturation and MDA were lower in non-splenectomized patients. However, this decrease was statistically significant for MDA only. The level of MDA in relation to desferoxamine therapy was also investigated, there was a lower serum MDA level in patients on desferal therapy (with regular or irregular) compared to patients not receiving chelating therapy. However, the difference was statistically not significant (p>0.05).

Correlations between serum MDA and serum zinc, copper and transferrin saturation parameters in thalassaemia major and minor patients were investigated. A statistically significant negative correlation (p<0.01, r=-0.55) was found between MDA generation and serum zinc levels in thalassaemia major patients only (Figure 1). How-

Table 1. Haematological findings in patients and controls.

Parameters	Control n = 142	β-thalassaemia Minor n = 116	β-thalassaemia Major n = 82
Hb gm/dl	12.4±1.5	11.1±1.8^{c***}	6.6±1.6$^{a*** b***}$
PCV %	40.4±3.7	36.0±4.2^{c***}	21.1±5.8$^{a*** b***}$
RBC Counts mil/µl	5.3±0.5	5.9±0.7^{c***}	3.3±0.9$^{a*** b***}$
MCV fl	78.5±3.6	69.6±3.9^{c***}	66.1±11.7$^{a*** b***}$
MCH pg	26.3±1.4	22.2±2.58^{c***}	20.9±4.5$^{a*** b***}$
MCHC %	32.6±1.9	30.5±3.9^{c***}	30.3±3.3^{a***}

Result expressed as mean ± SD

*** Significantly difference as compared with healthy subjects (p<0.001). a: Between major & control, b: between major & minor, c: between minor & control.

Table 2. Basic Biochemical characteristics in the studied groups.

	Control (n =142)	β-thalassaemia Minor (n =116)	β-thalassaemia Major (n = 82)
Iron (µg/dl)	112.7±21	124.5±19.1^{c***}	160.5±21.1$^{a*** b***}$
TIBC (µg/dl)	279.2±33.8	286.5±39.7	233.2±31.51$^{a*** b***}$
Trans. Sat %	38.2±7.1	43.9±6.7^{c***}	69.2±6.81$^{a***b ***}$
Ferritin (µg/l)	63.5±48	147.1±91.9^{c***}	1571.9±273.31$^{a*** b***}$
Zn (µg/dl)	93.9±27.4	88.6±24.7	47.7±14.11$^{a*** b***}$
Cu (µg/dl)	89.7±28.2	120.8±30.9^{c***}	154.1±28.51$^{a*** b***}$
Cu/Zn ratio	1±0.38	1.5±0.82^{c***}	3.45±1.47$^{a*** b***}$
MDA (µmol/l)	0.67±0.19	0.86±0.2^{c***}	1.14±0.31$^{a*** b***}$

Results were expressed as mean ±SD

*** Significantly different as compared with healthy subjects (p<0.001).

a: between major & control, b: between major and minor, c: between minor & control.

Table 3. Serum MDA levels in different age groups in patients with β-thalassaemia major and minor.

Age (years)	Group	MDA µg/l
1-6	Control n=20	0.66±0.09
	Minor n=13	0.73±0.08
	Major n=28	1.01±0.23^{a**}
7-12	Control n=23	0.66±0.12
	Minor n=15	0.72±0.06
	Major n=32	1.10±0.28^{a***,c**}
13-18	Control n=24	0.64±0.08
	Minor n=15	0.74±0.05
	Major n=11	1.30±0.49^{a***,c**}
>18	Control n=75	0.68±0.44
	Minor n=73	0.93 ±0.21^{b***}
	Major n=10	1.29 ±0.45^{a***,c*}

Result expressed as mean ±SD
*,**,*** Significantly different as compared with healthy subjects (p<0.05, p<0.01 & p<0.001 respectively)
a: between major & control, b: between minor & control, c: between major & minor.

ever, Figure 2 showed a statistically positive correlation between MDA generation and serum copper levels in both thalassaemia major and minor patients (p<0.01, r=0.52 and p<0.01, r=0.4 respectively). While, a statistically positive correlation (p<0.01, r=0.42) between MDA generation and transferrin saturation in thalassaemia major patients only was observed (Figure 3).

DISCUSSION

Patients with β-thalassaemia are subjected to a considerable degree of lipid peroxidation[10], which might influence the severity of the disease[2]. In the current study lipid peroxidation was explored by measurement of malondialdehyde (MDA), which is formed from the breakdown of polyunsaturated fatty acids, thus it serves as a convenient index for determining the extent of peroxidation reaction[18,19].

Lipid peroxidation was investigated in this study because of its effect on the degree of haemolysis, and because it is highly affected by the concentrations of iron, copper and zinc. Copper and iron can accelerate the auto oxidation of haemoglobin mainly due to their catalytic activity[5]. On the other hand, zinc acts as antioxidant as it is an integral part of the enzyme superoxide dismutase[2].

This study has illustrated a significant negative correlation of serum zinc levels and MDA production, and a significant positive correlation of serum copper and transferrin saturation with the production of MDA in β-thalassaemia major. In addition to that, serum MDA levels were

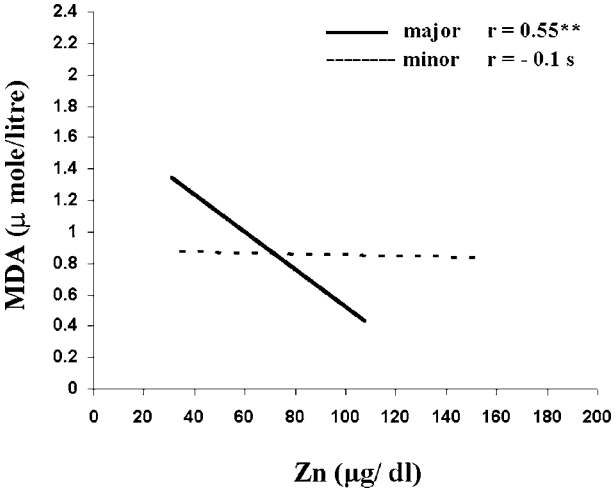

Figure 1. Correlation between serum zinc and MDA generation in β-thalassaemia major (n=82) and minor (n=116).

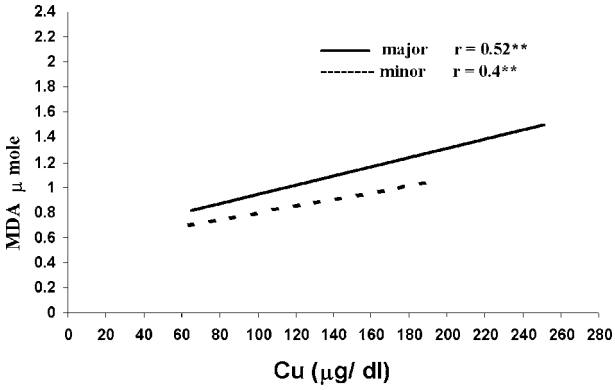

Figure 2. Correlation between serum copper and MDA generation in β-thalassaemia major (n=82) and minor (n=116).

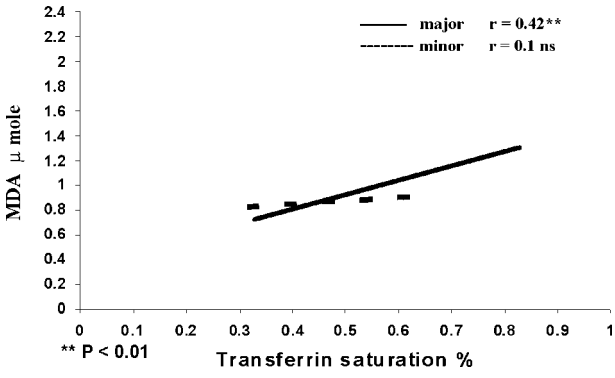

Figure 3. Correlation between transferrin saturation MDA generation in β-thalassaemia major (n=82) and minor (n=116).

significantly elevated in patients with β-thalassaemia major compared to control group. This result is in agreement with the results of Rachmilewitz *et al*[8], Suttnar *et al*[18] and Liveren *et al*[19]. These observations can be explained by the auto oxidation of unstable haemoglobin or isolated α-chains, which produces superoxide radicals (O_2^-) in addition to the presence of high erythrocyte iron and catalytic amount of copper[9-11]. Moreover, Rachmilewitz *et al*[8], revealed the interesting phenomenon of increased lipid contents per unit red cell, which makes it more susceptible to peroxidative threat. Furthermore, decreased haeme synthesis may lead to accumulation of non-haeme iron, in such circumstances, iron may act as a Fenton reagent, or it may catalyze the re-initiation of lipid peroxidation by breaking down the lipid hydroperoxide[5]. Recently, Kassab-Chekir *et al*[20] stated that their findings confirm the peroxidative status generated by iron overload in beta-thalassaemia major patients and highlight the rapid formation of marked amounts of thiobarbituric acid reactive substances (TBARS).

With regards to splenectomy, serum MDA levels was significantly higher in splenectomized than the non-splenectomized patients, which may be due to the role of spleen in moving the most pathological RBCs[18], therefore, in splenectomized patients more abnormal RBCs will exist in the circulation and these RBCs generate more MDA[14].

The effects of DFO therapy on MDA generation revealed that both groups with regular and irregular DFO therapy had lower serum MDA levels than those without therapy and this value was even lower in regular than in irregular therapy. It had been found in previous studies, in different experimental conditions that DFO efficiently inhibits lipid peroxidation and protects cells against oxidative damage[22,23].

In patients with β-thalassaemia minor, MDA generation was about 28.3% more than the control group, which was statistically significant. Almost the same result was observed by Vives Corrons *et al*[24]; who found that MDA generation is high in β-thalassaemia minor, indicating the susceptibility of the RBCs of this group to oxidative damage for similar reasons as for the β-thalassaemia major subjects but the severity is less.

Also in most of age groups of thalassaemia major patients, there were a significant higher levels of serum MDA as compared with the thalassaemia minor age matched groups, which could be due to that the severity of the disease is much higher in the homozygous type of the disease. However, the MDA generation in patients with β-thalassaemia minor in this study was only significantly correlated with serum copper concentration as serum copper has been shown to be significantly increased in β-thalassaemia minor patients as compared with control subjects.

From this study we may conclude that both β-thalassaemia major and minor have increased lipid peroxidation levels as detected by increased serum MDA. This finding suggests that thalassaemic RBCs are unlike normal one as they are most susceptible to auto-oxidation that may subsequently increase haemolysis and shorten the RBCs life span. Therefore, we recommend the implementation of MDA estimation, as it is a simple test for detecting lipid peroxidation. It is easy to perform and could be done to evaluate the degree of the disease severity since it could be used also for the management.

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