L–Carnitine ameliorates the iron mediated DNA degradation in peripheral leukocytes of β- thalassemic children.

Seham M. Ragab[1] and Riham G.Mahfouz[2]

[1]Departments of Pediatrics and [2]Biochemistry, Faculty of Medicine, Menoufiya University, Egypt.

ABSTRACT

Background: Iron overload is a common complication in β-thalassemia that induces intracellular oxidative stress producing lesions in the DNA including double strand breaks.

Objectives: The aim of this study was to evaluate DNA damage in peripheral leukocytes of β -thalassemic children and to investigate its association with the iron overload and the role of L-carnitine therapy upon this damage.

Subjects and Methods: Fifty β-thalassemic children (25 TM and 25 TI) with 20 age and sex matched apparently healthy children (control group) were included. Serum ferritin level was measured by ELISA. DNA damage was evaluated by the Gel electrophoresis to determine the total DNA genomic damage (TGD). The intensity of DNA nucleoprotein was measured by software Gel Pro analyzer computer program as maximum optical density (max.O D) values of apoptotic fragments of DNA at 200bp, 400bp and 600bp.

Results: The smear shape pattern on gel electrophoresis and Pro-Gel analyzer chart indicating double strand breakage of the DNA was detected in 76% of the thalassemic children. The thlassemic patients (the whole group and each of TM and TI groups) had significantly higher prevalence of DNA double -strand breaks in their leukocytes with significant higher values of max. OD at 200,400and 600 bp compared to the control group. The thalassemic children on regular L-carnitine therapy (50mg/kg/d for at least 6 months) had significantly lower prevalence and degrees of DNA breaks (TGD) with significant lower max. OD values at 200,400 and 600 bp compared to those not on L-carnitin therapy. There was significant positive correlation between the mean serum ferritin levels and the values of max. OD at 200 and 400bp. The data obtained from the Roc Curve shows that, the best sensitivity of 95% and specificity of 75% for the mean serum ferritin were at the cut off point of 820 ng/ml to predict the occurrence of TGD in thalassemic leukocytes.

Conclusions: Thalassemic children had significant DNA double-strand breaks in their leukocytes that was positively correlated to their iron overload reflected by serum ferritin level and can be ameliorated by L-carnitine supplementation.

Key Words:

β-Thalassemia, iron overload, oxidative stress, DNA damage, L-carnitine.

Corresponding Author:

Seham Mohammed Ragab, M.D.
E-mail: seham172001@yahoo.com

INTRODUCTION

β-Thalassemia is a hereditary anemia resulting from defects in the production of β-globin chains[1]. In β-thalassemia, various mutations result in absence (β^o) or reduced (β^+) synthesis of β-globin chains. Consequently, the relative excess of β-globin chains forms unstable tetramers which cause cellular damage[2]. Depending on clinical severity, two forms are distinguished, namely, thalassecmia major (TM) and thalassemia intermedia (TI).[3]

The oxidative status of cells is determined by the balance between pro-oxidants and antioxidants. Pro-oxidants, referred to as reactive oxygen species (ROS), are classified into radicals and non-radicals. ROS, when are generated in excess, may oxidize proteins, lipids and DNA-leading to cell death and organ damage. Oxidative stress is believed to aggravate the symptoms of many diseases, including hemolytic anemias.[4]

Chronic iron overload is currently considered to be the primary cause of morbidity and mortality of thalassemic patients. It results from a number of mechanisms associated with the disease itself. These mainly include ineffective erythropoiesis, peripheral hemolysis, increased intestinal iron absorption as well as repetitive blood transfusion[5]. In situations of iron excess, the iron-binding capacity of transferrin is saturated, resulting in the appearance of non-transferrin-bound iron (NTBI) in the plasma[6]. The toxicity of NTBI is attributed to its ability to catalyze free-radical reactions that have life-threatening consequences[7]. Iron could catalyze the oxidative breakdown of most biomolecules such as lipids, sugars, amino acids, DNA etc[8]. Iron-mediated lipid peroxidation, depletion of low-molecular weight antioxidants and single- and double-strand breaks in DNA have been implicated in the pathophysiology of iron overload diseases[9]. In humans, iron overload correlates with DNA alterations and cancer[10,11]. In thalassaemia patients, the high level of iron means that free radical generation is accessible, through Fenton-type chemistry. Therefore, these patients are particularly at risk of free radical induced damage including DNA strand breakage.[12]

In the treatment of thalassemia, newer approaches have been tried as alternative to standard therapy. Butyrate analogues such as L-carnitine have been found to increase HbF synthesis and hence used in treatment of β- thalassemia[13]. L-carnitine plays an essential role in fatty acid oxidation in mitochondria and energy production[14]. It also protects erythrocytes from oxidative stress, stabilizes the cell membrane, increasing the life span of red blood cells and is found to inhibit apoptosis in different diseases[15]. L-carnitine is a free radical

scavenger which prevents lipid peroxidation of cell membrane and apoptosis reducing serious tissue damage.[16]

The objectives of the present study were to evaluate DNA damage in peripheral leukocytes of β -thalassemic children and to investigate its association with the iron overload and the role of L-carnitine therapy upon this damage.

SUBJECTS AND METHODS

Subjects: This study included 50 β -thalassemic children regularly attending the pediatric hematology clinic in Menoufiya University Hospital for transfusion and chelation (25TM and 25TI). They were 25 males and 25 females aged 3-19 years (mean 9.7±4.44 years). Regarding TM patients they were 12 males and 13 females aged 3-19 years (mean 8.69±5.2 years). These patients were treated to maintain the pre-transfusion Hb level above 8 g/dl and post-transfusion Hb above 10 g/dl by regular red blood cell concentrates transfusion.

Thalassemia intermedia (TI) were 25 patients who regularly attend our hematology clinic for follow up and blood transfusion when needed. They were13 males and 12 females aged 4-18 years (mean 10.44±3.38 years). Twenty on transfusion (every one or two months) to maintain their growth and 5 were occasionally transfused. Twenty patients of TM group were under regular chelation by Desferroxamine (DFO) and 5 did not need to start the chelation yet. Of TI patients, 19 patients were regularly chelated by DFO and 6 did not need to start the chelation therapy.

To demonstrate the role of L-carnitine therapy, the study group was selected to involve a subgroup of patients under regular compliant L - carnitine therapy (50 mg/kg/day) for at least 6 months. This group included 10 TM and 10 TI patients.

Twenty age and sex matched apparently healthy children were enrolled as a control group. They were 9 males and 11 females aged 3 to 18 years (mean 9.1±3.79 years). Their Hb levels ranged from 12.0 to 14 g/dl (mean 13±0.5g/dl). All participants and their parents are informed with the study that was approved by the Ethics Committee.

Methods

Each participant was subjected to, full history taking and thorough clinical examination.

The mean yearly serum ferritin level in the previous year was considered (on the average of 3 determinations) for each patient. Serum ferritin was measured by Enzyme Linked Immune Sorbent Assay (ELISA) technique. Serum was prepared from a whole blood specimen without additives avoiding grossly hemolytic, lipemic, or turbid samples. Specimens were capped and stored frozen at 2-8^0 C till the time of the assay. 20µl of standard, sample or control were pipetted and added to 100µl of Enzyme Conjugate Reagent into all wells and thoroughly mixed for 30 seconds then incubated for 45 minutes at room temperature. Supernatant was decanted, washed 5 times with distilled or dionized water. 100µl of tetramethyl benzidine (TMB) Reagent were incubated for 20 minutes at room temperature in the dark. 100µl of the stop solution were pipetted and mixed gently for 30 seconds till the blue color changed completely to yellow. Absorbance was read at 450 nm within 15 minutes.

DNA fragmentation assay:

The blood sample was drown before blood transfusion. An amount of 1 ml of whole blood was incubated with 8 ml of (ELB). This is composed of 0.8 g ammonium chloride, 0.12 g sodium bicarbonate and 0.06 g EDTA dissolved in 100 ml distilled water. The blood was incubated for 10 minutes at 37^0 C. It was then centrifuged for 5 minutes at 1000 rpm. This step was repeated twice till a white pellet appeared[17]. The platelets were removed carefully. The leukocyte pellet was washed twice with RPMI 1640 medium, supplemented with 10% fetal bovine serum. The leucocytes were distributed in 15 ml sterilized falcon tubes at appropriate concentration (5x105 cells/ml) for treatment. DNA was extracted from both control's and patient's leukocytes based on salting out extraction methods according to the method of Al-Janabi and Martinez[18]. whereas protein was precipitated by saturated solution of NaCl, (5M).

Isolated leucocytes in eppendorf tubes were lysed by 600 microliter lysing buffer (50 mM NaCl, 1mM Na2 EDTA, 10% SDS pH 8.3) and was shaken gently. The mix was kept overnight at room temperature. For protein precipitation, an amount of 200 microliter of saturated NaCl was added to the samples and was gently shaken and centrifuged at 12,000 rpm for 10 minutes. The supernatant was transferred to new eppendorf tubes and the DNA was precipitated by 600 microliter cold iso-propanol. The mix was inverted several times till fine fibers of nucleic acids appeared and centrifuged for 5 minutes at 12,000 rpm. The supernatant was then removed. For washing, an amount of 500 microliter of 70% ethyl alcohol was added to pellet and decanted or tipped and the tubes blotted on whatman paper or clean tissue for 15 minutes. When the tubes were seen to dry, the pellets were resuspended in 50 microliter volume of TE buffer (10mM tris, 1 mM EDTA, pH 8) supplemented with 5% glycerol, gently pipetted and RNA was digested with Rnase for 1 hour.

Gel was prepared with 1.8% electrophoretic grade agarose (BRL). The agarose was boiled with tris -borate EDTA buffer (1 x TBE; 89 mM Tris, 89 mM boric acid, 2mM EDTA, pH 8.3). 0.5 microgram/ml ethidium bromide was added to the gel at 40^0C. Gel was poured and allowed to solidify at room temperature for 1 hour before samples were loaded.[19]

Gel Electrophoresis: Electrophoresis was performed for 2 hours at 50 volt in gel buffer (1 X TBE buffer) at room temperature with buffer level 2 mm cover the gel. Gel was photographed using a Polaroid camera while the DNA was visualized using a 312 nm UV transilluminator[20]. Electrophoresis pattern of nucleic acids determined total genomic damage (TGD) of DNA. The intensity of DNA nucleoprotein was measured by software Gel Pro- analyzer computer program (Ver 3.1). Apoptotic bands were located at 200 bp and its multiples. The intensity of apoptotic bands could be measured by Gel-Pro program as maximum optical density (Max.O D) values of apoptotic fragments of DNA at 200bp, 400bp and 600bp. The charts pattern denotes and confirms the degree of this total genomic damage (Fig. 1).

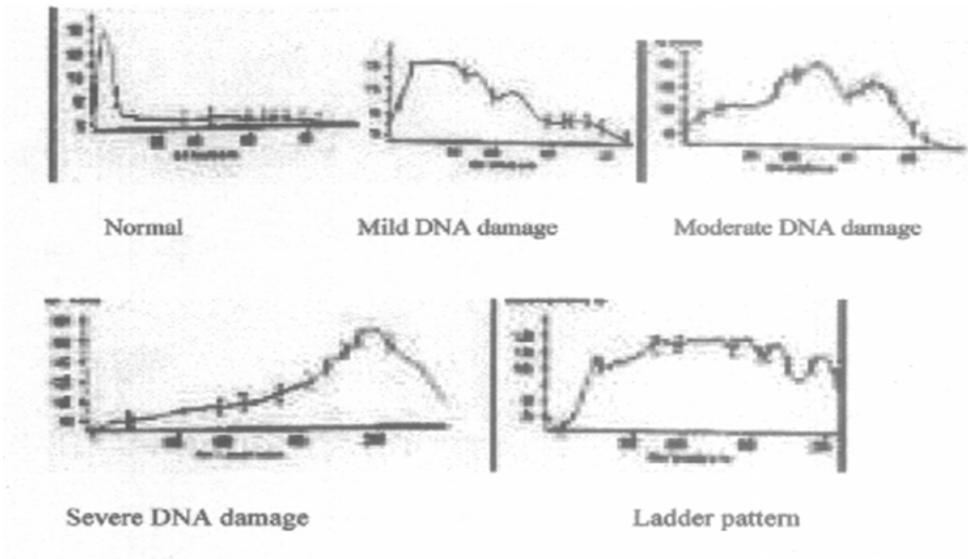

Fig. 1: Chart Patterns of DNA damage in the Gel –Pro analyzer.

Statistics: Results were collected, tabulated, statistically analyzed by IBM personal computer and Statistical Package for Social Sciences (SPSS) version 16. Differences between and within the groups were evaluated by the non-parametric Mann-Whitney test. Chi-square test ($\chi 2$) was used to study the relation between two qualitative variables. Pearson correlation (r): Is a test used to measure the association between two quantitative variables. Bivariate linear regression analysis test was used to study the power of certain risk factor. Receiver Operating Characteristic curve analysis (ROC curve) is a graph of sensitivity against 1- specificity at different cutoff points. The optimal cutoff point is that gives the highest sensitivity and specificity. P-value of <0.05 was considered statistically significant.[21]

RESULTS

The results of this study revealed that the mean yearly serum ferritin (ng/ml) was significantly higher in thalassemic children (as a whole) and in each of thalassmic groups (TM and TI) compared to the control group. There was no significant difference regarding the mean yearly serum ferritin between thalassemia major compared to thalassemia intermedia patients or between thalassemic patients on and those not on L-carnitine therapy (Table 1). Regarding the DNA damage, all children of the control group had normal electrophoretic pattern that was confirmed by normal charts of Pro-Gel analyzer program. No one of the control or the studied thalassemic children had the ladder pattern of apoptotic DNA damage. The smear shape pattern on gel electrophoresis indicating double -strand breakage of the DNA confirmed by the chart pattern of Pro-Gel analyzer was the main finding among thalassemic children (in 76%) mainly in the moderate and severe forms (Fig. 2). The thlassemic patients (the whole group and each of TM and TI Group) had significantly higher prevalence of DNA double -strand

breaks in their leukocytes demonstrated by the gel electrophoresis and Pro-Gel analyzer charts with significant higher values of max. O D at 200, 400 and 600 bp compared to the control group. There was no significant difference between TM and TI patients regarding the pattern of DNA double -strand breakage or the max. O D values at 200, 400 and 600 bp. The thalassemic children on regular L-carnitine therapy had significantly lower prevalence and degrees of DNA breaks (TGD) with significant lower max. O D values at 200, 400 and 600 bp compared to those not on L-carnitine therapy (Table 1). There was significant positive correlation between the mean serum ferritin levels and the values of max. O D at 200 and 400bp (Fig 3) without significant correlation to that at 600bp. The bivariate linear regression revealed that the mean serum ferritin predicted the value of max. OD at 200bp at B= 0.66 (P <0.001) while it was found to predict the value of max. OD at 400bp at B= 0.43 (P= 0.002). The data obtained from the Roc Curve shows that, the best sensitivity of 95% and specificity of 75% for the mean serum ferritin were at the cut off point of 820 ng/ml to predict the occurrence of TGD in thalassemic leukocytes, while at cutoff point of 980 ng/ml serum ferritin had sensitivity of 76% and specificity of 81% for DNA damage (Fig 4).

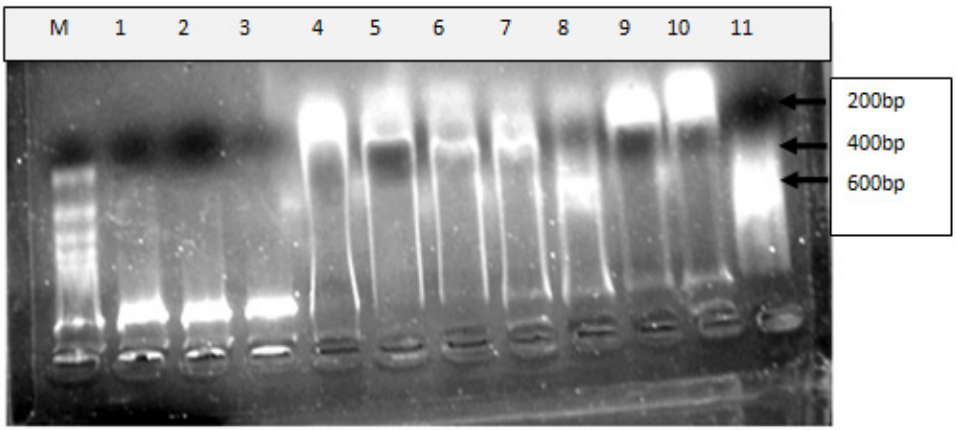

Fig. 2: Gel electrophoresis of some studied thalassemic patients and some control children.
M= Molecular weight marker.
Lanes 1, 2 and 3show intact DNA. Lane 1 for thalassemic child on L-carnitine therapy while lanes 2 and 3 for normal control children.
Lanes 4, 5, 6, 7, 9 and 10 show smear –shaped DNA damage of severe degree for thalassemic patients not on L-carnitin therapy.
Lane 8 shows smear shaped DNA damage of moderate severity for thalassemic patient not on L-carnitine therapy.
Lane 11 shows smear shaped DNA damage of mild severity for thalassemic patient on L-carnitine therapy.

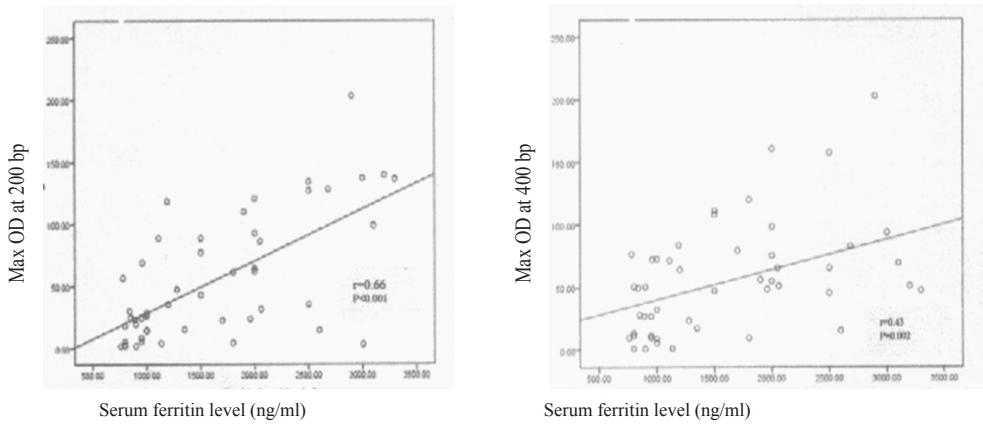

Fig. 3: Bivariate linear regression shows signifigant positive correlation between serum ferritin level and the max. OD at 200 and 400bp.

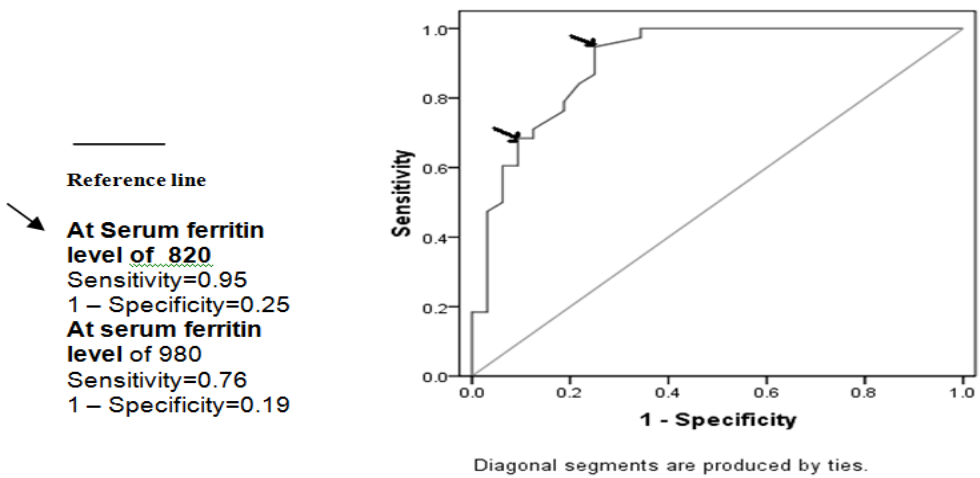

Fig. 4: The histogram represents the sensitivity and specificity of serum ferritin at different cut off points.

Table 1: Comparison between different groups regarding clinical characteristics, serum ferritin level, pattern of Electrophoretic DNA damage and maximum optical density values (Max.O D) of apoptotic fragments of DNA at 200bp, 400bp and 600bp:

Groups	Age $\bar{x}$ ±SD	Sex Male Female n % n %	Serum ferritin $\bar{x}$ ±SD	Pattern of the curve 1 2 3 4 n % n % n % n %	Max. OD at 200bp $\bar{x}$ ±SD	Max. OD at 400bp $\bar{x}$ ±SD	Max. OD at 600bp $\bar{x}$ ±SD
Thalaasemia (n=50)	9.7±4.44	25 50% 25 50%	1625±781	12 24% 8 16% 17 34% 13 26%	54.52±49.8	55.29±43.88	61.19±47.22
Control (n=20)	9.1±3.79	9 45% 11 55%	86.5±25.6	20 100%	8.73±14.88	14.53±24.01	19.26±26.7
P value	>0.05	>0.05	*<0.001	*<0.001	*<0.001	*<0.001	*<0.001
Thalassemia major (n=25)	8.69±5.25	12 48% 13 52%	1636±868	6 24% 5 20% 6 24 % 8 32%	57.23± 51.26	61.93±49.89	53±45
Control (n=20)	9.1±3.79	9 45% 11 55%	86.5±25.6	20 100%	8.73±14.88	14.53±24.01	19.26±26.7
P value	>0.05	>0.05	*<0.001	*<0.001	*<0.001	*<0.001	*<0.001
Thalassemia Intermedia (n=25)	10.44±3.38	13 52% 12 48%	1613±701	6 24% 3 12% 11 44 % 5 20%	51.83±49.24	48.66±36.47	69.38±48.85
Control (n=20)	9.1±3.79	9 45% 11 55%	86.5±25.6	20 100%	8.73±14.88	14.53±24.01	19.26±26.7
P value	>0.05	>0.05	*<0.001	*<0.001	*<0.001	*<0.001	*<0.001
Thalassemia Major (n=25)	8.69±5.25	12 48% 13 52%	1636±868	6 24% 5 20% 6 24 % 8 32%	57.23± 51.26	61.93±49.89	53±45
Thalassemai Intermedia (n=25)	10.44±3.38	13 52% 12 48%	1613±701	6 24% 3 12% 11 44 % 5 20%	51.83±49.24	48.66±36.47	69.38±48.85
P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Patients on L- carnitine (n=20)	10.12± 4.14	9 45% 11 55%	1570±813	10 30% 5 25% 3 15% 2 10 %	24.62±33.22	25.92±24.45	33.05±22.7
Patients without L-carnitine (n=30)	9.41±4.67	16 53.4% 14 46.6	1661±769	2 6.67% 3 10% 14 46.67% 11 36.66%	74.46±49.43	74.88±43.24	79.95±50.19
P value	>0.05	>0.05	>0.05	*<0.001	*<0.001	*<0.001	*<0.001

*= Significant.

1= Normal curve pattern. 2= Mild DNA damage pattern.

3= Moderate DNA damage pattern. 4= Severe DNA damage pattern.

Max. O D= maximum optical density values.

DISCUSSION

Oxidative injury is a major factor of accelerated ageing. It has been suggested that stress induces premature senescence, caused by rapid telomere shortening resulting from DNA damage including double-strand breaks[22, 23]. Thalassaemia patients are particularly at risk of free radical induced damage[24] caused by increased generation of ROS.[2]

Although increased oxidative stress and lipid peroxidation (LPO) in thalassemia patients have been clearly documented, the resulting DNA damage has not yet been extensively studied[25]. In this work DNA damage in peripheral leukocytes of thalassimic patients was investigated depending on the fact that circulating leukocytes are surrogate cells which continuously maintain a surveillance of the body for signs of toxic and antitoxic exposures[26]. The results of this study revealed that the smear shape pattern on gel electrophoresis indicating double stranded breakage of the DNA conferring fragmentation of extremely variable sizes (confirmed by the chart pattern of Pro-Gel analyzer) was the main finding among thalassemic children. It occurred in 38 out of the 50 studied patients (76%) mainly in the moderate (17 out of 50= 34%) and severe form (13 out of 50= 26%) (Table 1). The ladder pattern denoting apoptosis was not detected in any of our thalassemic patients or the control. All children of the control group had normal electrophoretic pattern. Compared to the control group, thlassemic patients (the whole group and each of TM and TI group) had significantly higher prevalence of DNA double -strand breaks in their leukocytes with significant higher values of max.O D at 200, 400and 600 bp.

In this regard Offer et al.[22] reported that relative to healthy control subjects, patients with thalassemia exhibited elevated levels of DNA damage as reflected by an increase in micronuclei containing RBCs.

Lymphocytes from thalassemic patients were reported to have higher levels of both background and induced DNA damage particularly DNA stand breaks as measured in a comet assay.[27]

Elevated LPO-induced adducted DNA was found in lymphocytes of thalassemic patients and in the liver of thalassemic mouse implicating that massive DNA damage occurs in the liver of these patients.[28]

Indirect assessment of DNA damage in thalassemic patients have been studied by Meerang et al[25]. who concluded that the strongly increased urinary excretion of etheno adducts in the studied thalassemic patients indicates elevated LPO-induced DNA damage in internal organs.

Also, thalassaemic patients had more sperm DNA damage than the controls ($P < 0.01$).[29, 30]

Iron plays an important role in oxidative tissue damage[31]. DNA has also been reported to be a target of iron-induced damage. Levels of some antioxidants are decreased during iron overload[32]. Iron overload is inevitable complication of thalassemia. About 73% of those born with thalassaemia could potentially develop iron overload and toxicity from transfusions and/or increased iron absorption.[8]

In this study serum ferritin was evaluated as a measure of the iron load in

the studied thalassemic children. Serum ferritin is the most commonly employed screening tool and can be used effectively to monitor changes in iron burden.[33] The mean serum ferritin level of the whole thalassemia group and that of each of TM and TI group were above 1000ng/ml. The studied thalassemic children (the whole group and each of TM and TI groups) had significant higher serum ferritin level compared to the control group (Table 1). We can conclude that significant DNA damage either TGD or the max.OD at 200, 400 and 600 bp in thalassemic children compared to the normal children could be attributed to that iron overload. In humans, iron overload correlates with DNA alterations and cancer.[10]

The results of this work had revealed significant positive correlation between the mean serum ferritin levels and the values of max. O D at 200 and 400bp (Fig. 3) without significant correlation to that at 600bp. The bivariate linear regression revealed that the mean serum ferritin was more correlated to the degree of DNA damage expressed by the value of max, OD at 200bp than at 400bp ($p < 0.001$ and $= 0.002$, respectively). This mean that iron overload expressed by high serum ferritin level is a risk for more severe degree of DNA strand breaks according to data interpretation from the Pro-Gel analyzer chart patterns (Fig. 1). In accordance to these results, Park et al.[34] had concluded that iron markedly induced DNA damage in humans and rat leukocytes, denoting that these white blood cells are sufficiently sensitive to assess exposure to iron and the measurement of DNA damage in human leucocytes could be used as a sensitive biomarker to study iron overload in vivo in humans.

Also, Almeida et al[35]. study results showed that iron-mediated lipid peroxidation was associated with intense mtDNA fragmentation. Perera et al[29]. reported positive association between the age of onset of chelation and sperm DNA damage and concluded that iron overload in thalassemia patients predisposes sperm to oxidative injury.

Serum ferritin concentration predicted the urinary excretion rate of 8-hydroxydeoxyguanosine a marker of oxidative stress, at B= 0.17 (95% CI 0.08-0.26, P= 0.001).[36]

In agreement of this, Naithani et al[37]. reported positive correlation between total plasma malondialdehyde (as a product of LPO) with serum ferritin levels of the studied thalassemic patients.

On the other hand Meerang et al[28]. and Cighetti[38] reported a negative correlation between plasma malondialdehyde and serum ferritin levels in the studied patients with thalassemia.

Iron overload is generally defined as serum ferritin consistently >1000 ng/ml[39]. In addition, the critical level of iron overload at which toxicity and organ dysfunction occur, or the 'tipping point', has not been established definitively for serum ferritin[40]. The result of this work revealed that at cutoff point of 820 ng/ml serum ferritin has sensitivity of 95% and specificity of 75%, for DNA damage while it has sensitivity of 76% and specificity of 81% for DNA damage at cut off point of 980 ng/ml (Fig. 4).This means that DNA strand breaks occurred at serum ferritin levels below and around the defined level of iron overload supporting the importance of good chelation therapy for thalassemic children aiming at keeping their serum

ferritin level below 1000ng/ml. The previous result came in accordance with was reported and recommended that serum ferritin >1000 ng/ml on repeated measurements has been proposed as the threshold for initiation of iron reduction therapy in transfusion-dependent patients.[41, 42]

Comparison between the studied thalassemia major and thalassemia intermedia patients had revealed non significant difference between the two groups in the electrophoretic pattern of DNA damage or in the values of max.OD at 200, 400 or 600bp (Table 1). This could be attributed to the non significant difference in the iron load reflected by serum ferritin level between them.

DNA damage and unrepaired strand breaks are associated with age-related diseases[43]. Carnitine has been categorized as a conditionally essential nutrient and was found to be beneficial in the treatment of deficits associated with the aging process[44]. During the last years, L-carnitine was introduced as a safe and effective adjunctive therapeutic approach in thalassemic patients.[45]

In this work a subgroup of thalassemic children under regular L-carnitine therapy (50mg/kg/d) for at least 6 months was included. The mean yearly serum ferritin level was lower in those on than those not on L-carnitine therapy (1570±813 versus 1661±769 ng/ml, respectively), yet the difference did not reach a significant level. The thalassemic children on regular L-carnitine therapy had significantly lower prevalence and degrees of DNA breaks (TGD) with significant lower max. OD values at 200, 400 and 600 bp compared to those not on L-carnitine therapy without significant difference in serum ferritin level (Table 1). These results denotes that with a matched iron load reflected by serum ferritin level, L-carnitine therapy had a significant protective effect against DNA fragmentation.

In agreement with the results of this work, previous studies have demonstrated that carnitine reacts with superoxide and hydrogen peroxide, has reducing properties, chelates iron and prevents lipid peroxidation and DNA cleavage[46,47] L-Carnitine caused a decrease in single strand breaks in human peripheral blood lymphocytes induced by oxygen radicals and alkylating agents.[48] In aged animals, administration of L-carnitine for 21 days significantly decreased the levels of lipid peroxides and improved the activities of antioxidant enzymes. It significantly reduced DNA damage, apoptosis in lymphocytes of these animals.[49] The therapeutic potential of L-carnitine may be related to its antiapoptotic properties that had been documented in several studies.[50-53]

Also, the protective effect against DNA damage in thalassemic children documented in this work comes in accordance with and can give an explanation for the confirmed beneficial effect of L-carnitine supplementation in thalassemia patients on several hematological and other parameters of health and wellbeing that were reported in several studies.[54-57]

In conclusion, thalassemic children had significant DNA double-strand breaks that was positively correlated to their iron overload reflected by serum ferritin level and can be ameliorated by L-carnitine supplementation. Hence, good chelation keeping the serum ferritin

level below 1000ng/ml and L-carnitine supplementation are recommended for thalassemic children.

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