Thalassemia Major: Effect of transfusional iron overload on liver, kidneys functions and antioxidant status of B-thalassemic patients in Wasit Governorate

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Abstract: Thalassemia is the commonest form of heamoglubinpathy in the world, in which patients may need frequent regular blood transfusion that may lead to impairment the normal function of many organs such as heart, liver, kidneys, and endocrine glands due to increase free iron deposition in these vital organs. The present study was carried out to investigate the effect of transfusional iron overload on the liver and kidney functions and on the oxidative stress status of the patients with B-thalassemia major and compared the results with healthy volunteer subjects (control group). Forty six patients with B-thalassemia major and forty healthy individuals were used in the current study, the patients were analyzed at thalassemia center of Al-Kut hospital to assessment the liver and kidney functions via measure the alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), albumin, creatinine, urea and serum ferritin and the correlation between serum ferritin and ALT concentration. In addition, assess the antioxidant status of the thalassemic patients by measure the serum malondialdehyde (MDA), Catalase and Reduced glutathione (GSH). The results have been shown significant increase ($P \leq 0.05$) in all the above criteria related with liver and kidney function (ALT, AST, ALP, Ferritin creatinine), as well as diminished antioxidant status via increased MDA and decreased catalase and GSH. In addition , serum ferritin concentration revealed potent correlation with serum ALT and MDA concentration . Therefore, the results explain that regular blood transfusion to the B-thalassemia major patients (despite increase lifespin of patients) lead to increase deposition of iron in kidney and liver causing iron overload despite of the patients received iron chelating therapy to reduce accumulation of iron.

Keywords: major thalassemia, Iron overload, Oxidative stress, blood transfusion.

Introduction

Thalassemia is the group of genetic defect of hemoglobin synthesis, it is manifested by impairment of synthesis one or more of globulin chain of hemoglobin (1). According to the disorder that take place in globulin chain, thalassemia classified into α-thalassemia and β-thalassemia , and the last one subdivided into β-thalassemia minor β-thalassemia intermediate and β-thalassemia major which is the most severe form of β-thalassemia that characterized by sever hemolysis of peripheral circulation and the patients should be treated with blood transfusion .(2 ,3). The main cause of morbidity and mortality of most thalassemic patients disorder of liver , heart , kidneys, and endocrine glands (4 ,5

,6). Hemoglobinpathy of β-thalassemia major (BTM) lead to chronic anemia and hemolysis , in effective erythropoiesis and transfusional iron overload (7). The liver is the main site of iron storage and the only site for synthesis of ferritin. Unbound iron is very toxic to the tissues and normally present bound with protein in the liver , therefore, in effective of erythropoiesis is the main cause of anemia and responsible for increased erythropoietic demand, leading to excessive intestinal iron absorption (8,9). Iron overload also occur due to regular blood transfusion in the individuals have been suffered from β-thalassemia major , subsequently , lead to cardiac and hepatic failure and premature death (10).

On the other hand, anemia and hypoxia associated with oxidative stress that may accelerate the decline renal functions by chronic hypoxia of renal tubular cell with increased metabolic demand, causes development of tubule-interstitial injuries and glomerulo-sclerosis and kidney fibrosis (11, 12). Chelating therapy is aimed to reduced iron burden by removing excess iron from body, these chelating agents very effective treatment of transfusional iron overload that lead to extend patients survival (13,14). Although of these beneficial effect of chelating therapy, it has been reported that the iron chelator such as deferoxamin (DFO) can cause renal tubular dysfunction (15,16). Therefore , this study performed to determine the effect of regular blood transfusion in B-thalassemia patients on some physiological parameters comparing with healthy subjects.

Material and methods

This study was carried out in the thalassemia center in Al-Kut hospital from 26/11/2016 to 26/2/2017 . Eighty six individual were used in the current study, forty health volunteers persons, and forty six patients with B-thalassemia major (BTM) that diagnosed depend on the results of hemoglobin electrophoresis and hematological profile. Those patients received desferal drug as chelating therapy more than one years , and all patients were tested and found free from hepatitis B and C that were received regular blood transfusion, usually they were given every 2 to 4 weeks. Five ml of venous blood samples were drown into sterilized tube from each individual. The samples were centrifuged at 3000 rpm for 15 minutes at 25 °C and the serum sample were stored at 4°C until needed for biochemical testes related with liver and kidneys function, that include serum ferritin level , ALT, AST, ALP, albumin , creatinine and urea in addition estimation of antioxidant status by measuring the malondieldehyde (MDA), Catalase and reduced glutathione (GSH).

Biochemical Testes

Serum alanine aminotransferase (ALT):

Serum ALT activity was enzymatically measured using standard assay (Biomagreb chemical-kit) based on the fact that glutamic transaminase enzyme, catalyze the transfer of the amino group of glutamic acid to pyruvic acid in reversible reaction (17).

serum aspartate aminotransferase (AST).

Serum AST activity was enzymatically measured using standard assay (Biomagreb chemical-kit) based on the fact that glutamic transaminase enzyme, catalyze the transfer of the amino group of Aspartic acid to 2-Oxoglutarate acid in reversible reaction (17).

Alkaline Phosphatase

Alkaline Phosphatase Assay Kit is designed to measure ALP activity. The method utilizes p-nitrophenyl phosphate that is hydrolyzed by ALP into a yellow colored product (maximal absorbance at 405nm). The rate of the reaction is directly proportional to the enzyme activity (18)

p -nitrophenol phosphate $\xrightarrow{ALP}$ p -Nitrophenyl + phosphate

Serum creatinine

serum creatinine concentration was esstemated by (19) in which the creatinine reacts with picric acid at alkaline pH to form a yellow orange complex.

Serum albumin concentration (g\dl)

Estimation of serum albumin concentration was depend upon the method of Doumas etal., (20) which based on the specific binding of bromocresol green (BCG) and the protein in acidic PH, with resulting shift in the absorption wave length of the complex. The intensity of the color formed is proportional to the concentration of albumin in sample .

Serum urea concentration

In the assay,(using BioVision's Urea Assay Kit) Urea reacts as substrate with compounds in the presence of enzymes to form a product that reacts with the OxiRed probe to generate color (λmax=570nm). The optical density of produced color has a direct relationship with Urea concentration in the solution. The kit can detect as low as 0.5 nmol per well or 10 µM of Urea. The assay is also suitable for high throughput studies.

Measurement Antioxidant status

The antioxidant enzymes were assayed in serum, serum reduced glutathione concentration (GSH) Detemined spectrophotometrically at 412 nm and is directly proportional to the GSH concentration according the method of **Burtis, C. and Ashwood** (21) . The CAT activity was measured as described by the method of Claiborne (22) , Assessment malondialdehyde (MDA) concentration to determined lipid peoxidation By using the thiobarbituric acid (TBA) that is read at 535nm (23)

Statistical Analysis

The statistical analysis of data was performed by using Independent T-test was used to determine statistical difference in the mean values between study and control group . A p value of < 0.05 was considered statistically significant (24).

Results and Discussion

Kidney and liver enzyme

The concentration of ALT, AST, ALP, serum albumin concentration, serum creatinine concentration, serum urea concentration and serum ferritin level in all subjects were clarified in table and figure (1). Depending on the results , there were significant (P< 0.05) increase were recorded in the mean values of ALT, AST, ALP, serum creatinine and serum ferritin level comparing the values of healthy volunteers, while

Table (1): Biochemical changes in serum level of liver and kidney diagnostic parameters

Group / Parameter	Healthy subjects (control)	Thalassemic patients
ALT (U/L)	32.33 ± 1.46	46.35 ± 1.85 *
AST (U/L)	30.64 ± 1.73	40.43 ± 1.93 *
ALP (U/L)	57.80 ± 3.45	84.26 ± 5.67 *
Albumin (g\dl)	47.25 ± 1.85	45.60 ± 2.34
Creatinine (mg/dl)	0.965 ± 0.12	1.624 ± 0. 33 *
Urea (mg/dl)	26.88 ± 1.37	28.11 ± 1.89
Ferritin (µg/L)	112.65 ± 22.73	2149.57 ± 149.82*

Values are presented as mean ± SE.M; * Significant different from control (P<0.05) using independent t-test

there was no significant difference (P> 0.05) in the mean values of albumin and urea between the studied groups.

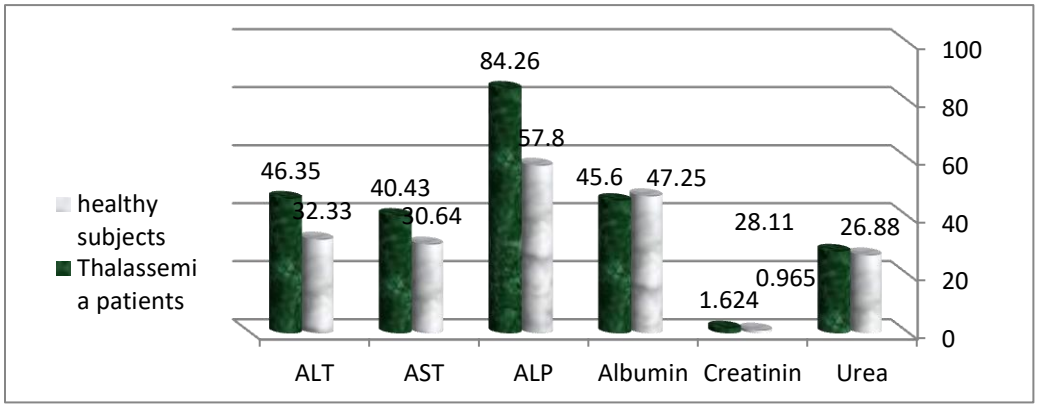

Figure (1): The differences in mean values of liver and kidney functions parameters

Antioxidant status

Table (2) demonstrated the mean value of Malondialdehyde (MDA), Catalase and reduced glutathione (GSH). The results clarified the significant (P< 0.05) differences in the mean value of all oxidants biomarkers , in which there were significant elevation in the MDA concentration comparing with the group of healthy subjects, as well as, there were significant (P< 0.05) decline in the serum concentration of catalase and GSH in thalassemia patients compared with control group.

Table (2): Effect of Transfusional iron overload on serum antioxidant status

Parameter / Group	Malondialdehyde (**nmol/ml)**	Catalase **U/mg**	Reduced glutathione GSH (nmol/ ml)
Healthy subjects	2.12 ± 0.132	7.65 ± 2.17	8.08 ± 1.13
Thalassemic patients	3.32± 0.158 *	4.67 ± 2.33 *	2.66 ± 3.82 *

Values are presented as mean ± SE.M; * Significant different from control (P<0.05) using independent t-test

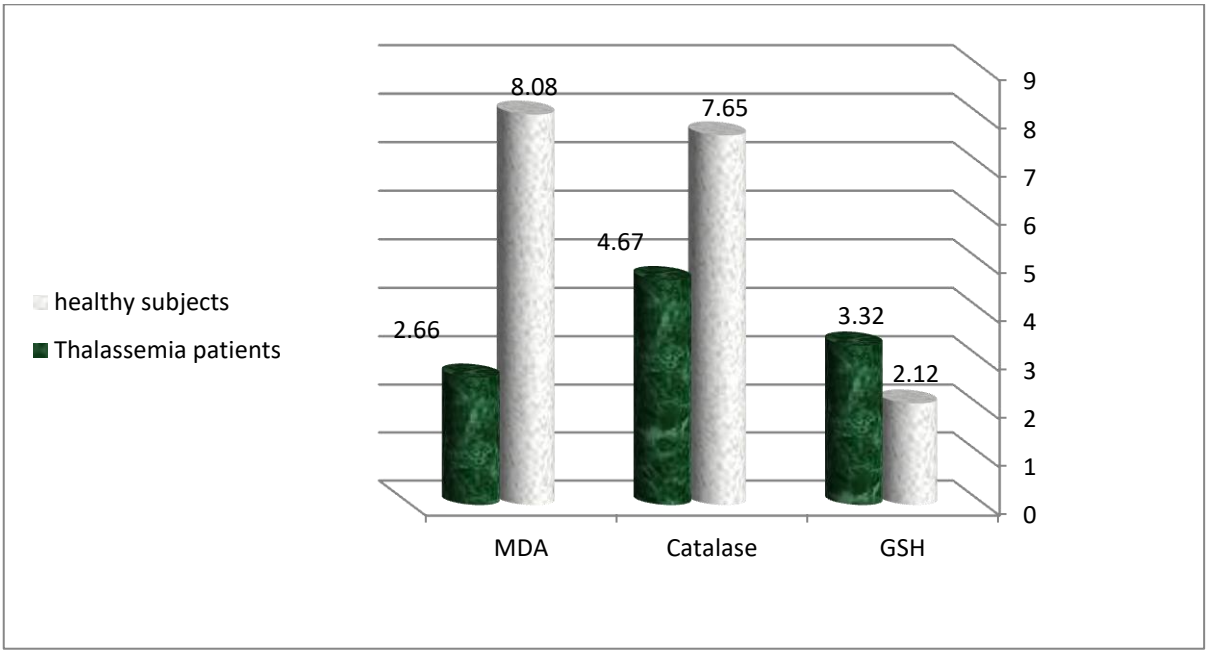

Figure (2): Oxidant related enzyme in healthy subjects and thalassemia patients

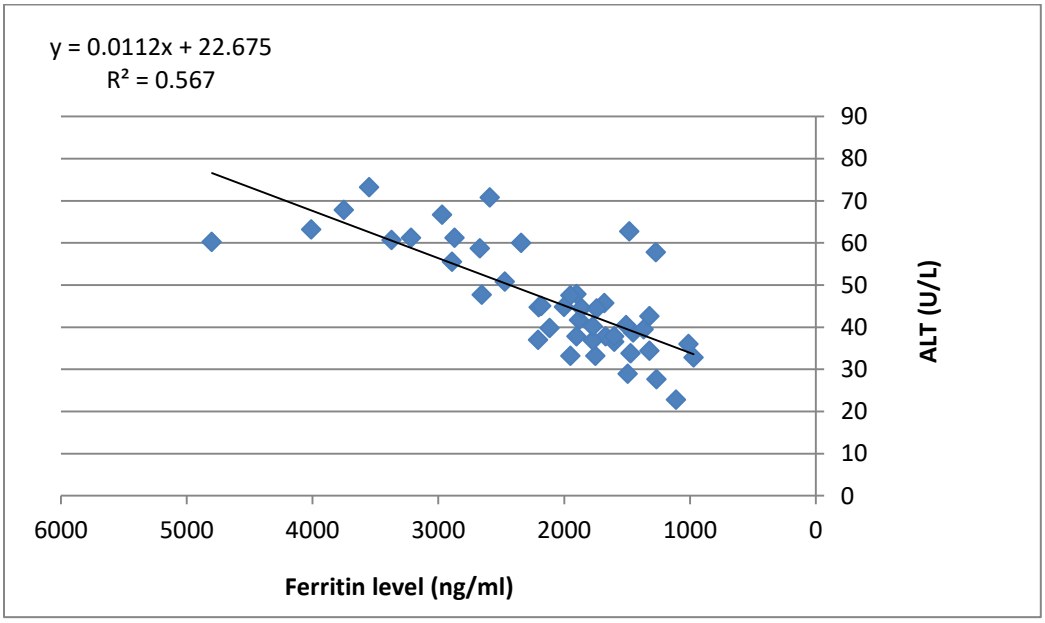

Figure (3) : Relationship between Serum Ferritin level and Alanine aminotransferase (ALT) in B-thalassemia patients .

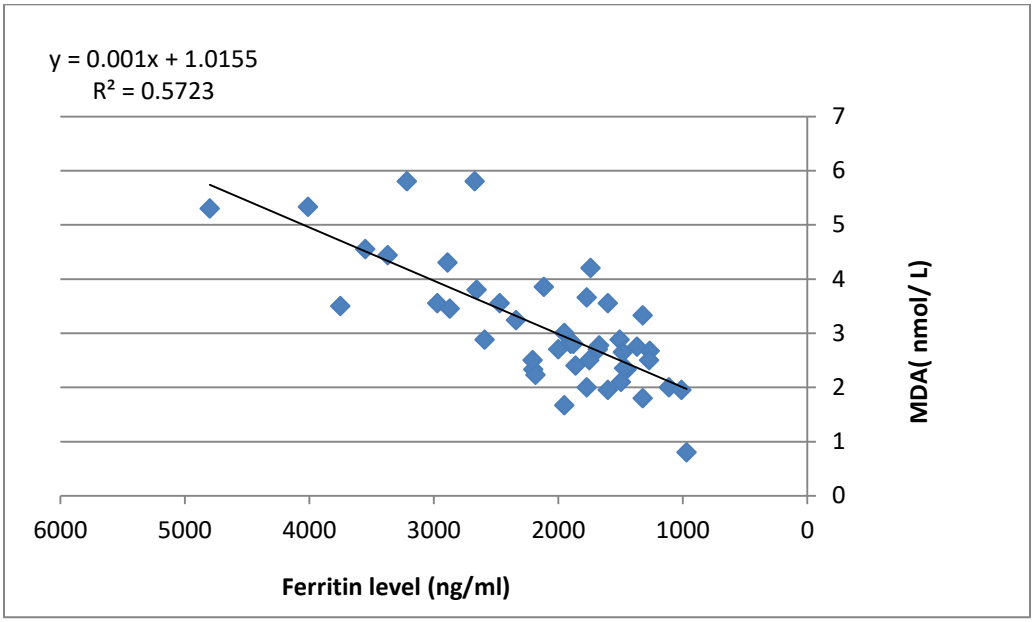

Figure (4): Relationship between Serum Ferritin level and Malondialdehyde (MDA) in B-thalassemia patients .

Liver enzymes is relatively related with liver cell damage that caused by toxic effect of iron deposition in hepatocyte and oxidative injury of liver cells by free radicals (25). The results of current study agreement with the finding of several studies whose they shown increase of serum ferritin lead to increase of liver enzymes as ALT, AST, and ALP in homozygous thalassemia patients (26,27). The liver is the more organ that affected by iron overload by its deposition in both hepatocytes and reticulo-endothelial cells, which may cause liver injury and development of fibrosis and cirrhosis (25).thus, there were positive correlation between increase ferritin level and ALT concenteration (figure3). Iron overload lead to accumulation of iron in the kidneys and subsequently induce release of free reaction iron which stimulation the production reactive oxygen species (28, 29). The effect of iron deposition on kidneys function was proven by (30) who revealed increased serum cystatin-C (indicator of glomerular dysfunction) in the serum of BTM.

Recent study showed that regular blood transfusion to BTM lead to deposition of hemosiderin in visceral and parietal glomerular epithelial cells, as well as , the correlate positively between the serum ferritin level and liver iron deposition (31) . also, iron chelating agents that given to the BTM to decrease the level of iron may cause glomerular dysfunction which may lead to increase serum creatinine , this fact was proven by many studies that clarified increase the serum creatinine and acute kidney injury take place in the patients with BTM given chelator agents (32, 33). The regular blood transfusion along with chelating agents improve the quality and duration life, but iron overload is a serious complication of long term blood transfusion with increase production of free radicals and peroxidative damage of tissue. Normally the intracellular antioxidant prevent damage of free radicals and increase release of heme has been shown to directly inhibit a number of cytoplasmic enzyme (34). Glutathione is the most important

antioxidant that present in the cellular and subcellular membrane that has ability to protect the cell and tissue from damage by catalyzing the reduction of lipid hydroperoxide via the enzyme glutathione peroxidase (GPx), therefor the GPx value close related with glutathione level so lower of GPx with thalassemia children due to increase peroxidative damage and decrease antioxidant enzyme (35)

On the other hand the result revealed significant increase in MDA in the patients with BTM received regular blood transfusion. MDA a biomarker of oxidative stress these results agreement with study that clarified the oxidative stress caused by free iron play essential role in cytotoxic effect of epithelial tubular cell lead to increase glomerular infiltration (36).Cytotoxicity occurs by reduction cellular energy production lead to decrease ATP (37). As well as, recent studies showed that iron overloaded BTM patients lead to formation of toxic oxygen species , causing oxidative lipid peroxidation and increase thiobarbituric acid reactive substance and depletion in antioxidant cellular mechanism (38, 39). Also the results clarified there were a positive correlation between the level of MDA and serum ferritin level in thalassemic patients (figure 4).

Conclusion : The results of this study investigate that regular blood transfusion, despite of increase life duration, can cause transfusional iron overload that can lead increase free radical and peroxidative damage of cellular membrane which impairment the function of many vital organ like liver and kidney.

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