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Association of the changes in hepatic enzymes, bilirubin, and plasma proteins with beta-thalassemia in iron over loaded-patients

Ghufran Mohammed Hussain[1,*], Maytham Ahmed Abdullah[2], Nibras Yahya Hussein[3]

[1]College of Pharmacy, AL-Zahraa University for Women, Karbala, Iraq
[2]College of Pharmacy, Al-Mustaqbal University College, Babylon, Iraq
[3]Department of Pharmacy, Al-Manara College for Medical Sciences, Amarah, Iraq

***Corresponding author**
Ghufran Mohammed Hussein
College of Pharmacy, AL-Zahraa University for Women, Karbala state, 56001, Iraq
e-mail: ghufran.mohammed@alzahraa.edu.iq

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ABSTRACT

Changes in liver enzymes and bilirubin in patients with thalassemia depend on genetic changes and the association of other genetic determinants. Iron overload is associated with increased morbidity in both transfusion-dependent and non-transfusion-dependent thalassemia patients. The main objective was to evaluate hepatic enzymes, bilirubin, and plasma proteins in beta-thalassemia major (βTM) patients and to study the correlations of these parameters with serum hemoglobin and ferritin concentrations in βTM patients. To achieve these, the study had two groups, the first is case group includes 39 patients with βTM and the second control group includes 34 subjects. Serum ALT, AST, total bilirubin, albumin, and total protein concentrations were measured by UV-Vis spectrophotometer, while the concentration of serum ferritin was measured by ELISA Kit. Our results showed that there were highly significant differences between βTM and ferritin, furthermore, there were highly significant differences between βTM and liver enzymes such as AST, ALT, total protein, albumin, and total bilirubin. In addition, our findings showed that there was correlation between serum ferritin concentrations and liver function parameters in βTM patients, where there are highly significant differences between βTM and ALT, while the correlation between serum hemoglobin concentration and liver function parameters showed highly significant differences between βTM and AST. The study concluded that the increase in serum liver enzymes (ALT, AST) and total bilirubin concentrations in patients with βTM are indicator to liver dysfunction that is correlated to iron overload.

INTRODUCTION

Thalassemia is a genetic disease that produced from insufficient or absent in synthesis of globin chains (alpha or beta) in hemoglobin [1]. Beta-thalassemia resulted from a defect in production of beta globin chain and classified into beta-thalassemia minor, intermediate and major [2, 3]. Beta-thalassemia minor caused by defect in a single beta globin chain and the patient is asymptomatic [4]. Beta-thalassemia intermediate which is an intermediate state between beta-thalassemia minor and major, where patients may live a normal life, but need occasional blood transfusion in times of illness [5]. Beta-thalassemia major (βTM) caused by defect in a two-beta globin chain and the patient is symptomatic, suffers from severe anemia, and to live normally, the patient required a regular blood transfusion [6]. Patients with thalassemia are important clinical diseases, and there are two types of thalassemia, alpha and beta [7]. Where the proportion of hemoglobin (Hb) varies in patients with beta thalassemia type, as a result of the decrease or absence of the production of the hemoglobin-chain of Hb tetra hemoglobin (+) or (β0), respectively, which leads to a decrease in the normal hemoglobin in red blood cells (RBC) [8]. Recurrent transfusions of blood increase the life expectancy and

improve the life quality for the beta-thalassemia major patients, but it causes iron overload, that is a main treatment complication [9]. Iron overload is the consequences of blood transfusions and increase of iron absorption from intestine. The deposition of iron in the liver, heart and endocrine glands causes severe damage to these organs [10-12]. The aims of the study are to evaluate hepatic enzymes, bilirubin, and plasma proteins in βTM patients and to study the correlations of these parameters with serum hemoglobin and ferritin concentrations in βTM patients.

MATERIALS AND METHODS

Sample collection

The study involves two groups, the first group is βTM patients. This group includes thirty-nine patients with βTM (the age ranged from 5 to 17 years), who attended the Babylon Maternity and Pediatric Hospital (thalassemia center) in Hilla city. The second group is the control group (apparently healthy). This group includes thirty-four subjects (the age ranged from 5 to 16 years).

This research was conducted based on the ethical approval issued by the Ministries of Health and the Ministry of Higher Education and Scientific Research in our country, numbered (DSM / HO-15314) on 16/4/2022 for scientific research, with all informed consents taken before taking and collecting samples and conducting experiments by the committee.

Biochemical analysis

About 5mL of blood were drawn by vein puncture from all subjects. The collected blood samples were divided into two parts, the first part (3 mL) put in plain tube for measuring of serum ferritin, alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, albumin, and total protein concentrations, while the second part (2 mL) put in EDTA tube for measuring of serum hemoglobin concentration and hemoglobin-electrophoresis analysis. Serum ALT, AST, total bilirubin, albumin, and total protein concentrations was measured by UV-Vis Spectrophotometer (Shimadzu/Japan) and the analysis was carried out according to the manufacturer's instructions. Also, the concentration of serum ferritin was performed based on the manufacturer's instructions, an enhanced high-quality Elabscience® ELISA (Nanjing Pars Biochem (CO.,Ltd/China)) that allows to target serum ferritin with high accuracy.

Statistical analysis

The statistical analysis of the obtained results was carried out using the SPSS program (version 23) through several tests, including (ANOVA) and t-test. The probability level was also found at P-value < 0.05 [13].

RESULTS

Demographic characteristics and liver functions in βTM patients

Table 1 showing the demographic characteristics of βTM and control groups, where there were highly significant differences in ferritin concentrations (2942.71 µg/L) in βTM patients. Furthermore, there was a decreased mean age at 10.71 years in βTM patients, and decreased levels of the mean hemoglobin (6.82 g/L) as shown in Table 1.

Table 2 showing the liver function parameters of βTM patients and control groups, where there were a significantly higher levels of AST (71.98 IU/L) in βTM patients as compared to control. Also, there were a significantly higher levels of ALT (69.42 IU/L) and total bilirubin (2.32 mg/dL). However, there were a lower level of total protein (6.98 mg/dL) and albumin (3.92g/dL) in βTM patients as compared to control (Table 2).

Table 1. Demographic characteristics of βTM and control groups.

Character	Group	Mean ± SD	P value
Number	Control	34	
	βTM	39	
Sex [Male/Female]	Control	19/15	
	βTM	22/17	
Age [year]	Control	11.25 ± 3.50	P < 0.05
	βTM	10.71 ± 3.08	
Hemoglobin [g/L]	Control	12.19 ± 1.57	
	βTM	6.82 ± 1.33	
Ferritin [µg/L]	Control	63.83 ± 21.62	
	βTM	2942.71 ± 1512.25	

Table 2. Liver function parameters of βTM patients and control groups.

Parameter	Group	Mean ± SD	P value
ALT [IU/L]	Control	23.92 ± 7.45	
	βTM	69.42 ± 23.72	
AST [IU/L]	Control	21.22 ± 6.91	
	βTM	71.98 ± 21.45	
Total bilirubin [mg/dL]	Control	0.58 ± 0.11	
	βTM	2.32 ± 0.91	P < 0.05
Albumin [g/dL]	Control	4.02 ± 0.42	
	βTM	3.92 ± 0.39	
Total protein [g/dL]	Control	7.34 ± 5.92	
	βTM	6.98 ± 6.42	

Correlation between serum ferritin and liver function parameters

Figure 1 showing the correlations of serum ferritin concentrations with liver function parameters in βTM patients, where there was a highly significant correlation in ALT (r=0.44) and AST (r=0.39) levels in βTM patients. However, there was a lower correlation in total protein (r = 0.14), total bilirubin (r = 0.13), and albumin (r = 0.08) in βTM patients.

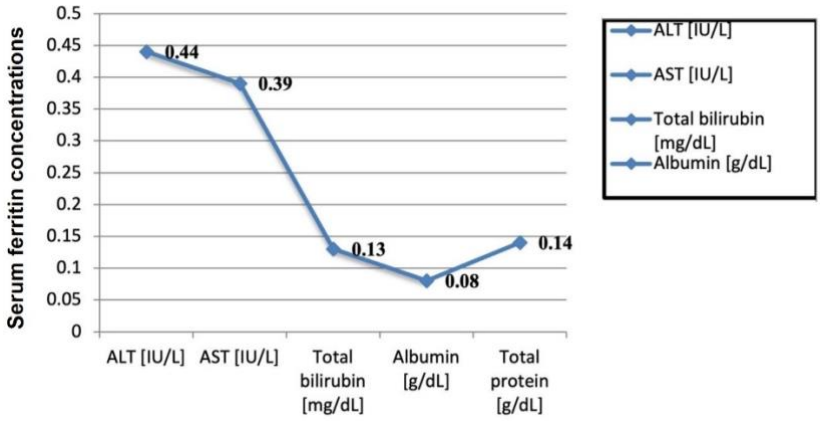

Figure 1. Correlations of serum ferritin concentrations with liver function parameters in βTM patients.

Correlation between serum hemoglobin and liver function parameters

Figure 2 showing the correlations of serum hemoglobin concentrations with liver function parameters in βTM patients, where there was a highly significant correlation in ALT (r=0.41) and AST (r=0.52) levels in βTM patients. However, there was a lower correlation in total protein (r = 0.09), total bilirubin (r = 0.15), and albumin (r = 0.10) in βTM patients.

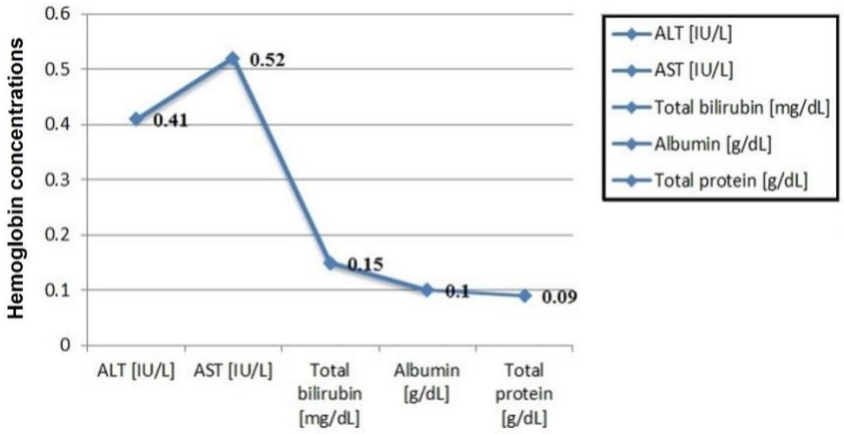

Figure 2. Correlation of serum hemoglobin concentration with liver function parameters in βTM patients.

DISCUSSION

The patients of βTM suffering from defect in a synthesis of beta globin chains of hemoglobin that leads to red blood cells damage and destruction in bone marrow and hemolysis in peripheral circulation. For this reason, our study aimed at the correlation between some liver enzymes, bilirubin, and hemoglobin in the blood to identify the effect of the disease on the concentrations of these biochemical parameters [14, 15]. In addition, the beta-thalassemia major is associated with iron overload that resulted from many causes which are frequent blood transfusions, ineffective erythropoiesis, peripheral hemolysis and increase the iron absorption from intestinal [16-18].

Our results showed a significant increase in ferritin and low level of hemoglobin, as shown in Table 1, and this is consistent with what many studies indicated that excess iron in thalassemia patients is highly toxic to all tissues of the body and causes serious and irreversible biological damage to many organs such as the liver and its enzymes, the heart, endocrine glands, and blood proteins such as ferritin, which increases their concentrations in the blood [20].

The results of the present study showed a significant increase in the mean level of serum ferritin (have important role to store iron) of beta thalassemia patients in comparison with the control group (Table 1), this is because excess iron may cause a rise in blood ferritin levels [21]. These results may agree with many studies that mentioned that the body has some mechanisms to absorb, store or transport iron, but there is no mechanism for iron secretion outside the body, which increases ferritin concentrations in the blood [22]. In addition, iron concentrations are increased in patients with βTM in response to ineffective erythropoiesis by absorption of more iron from the diet [23]. The increasing of serum ferritin concentration in βTM patients reflects the iron overload state that resulted from erythrocyte hemolysis and/or blood transfusion. Also, the result shows a decrease in hemoglobin concentration that resulted from the erythrocyte hemolysis, these results similar the results with the

researchers Karim *et al* and Larson *et al* Those who mentioned that the high level of ferritin in patients with beta thalassemia results from the large storage of iron. On the other hand, there is an increase in the breakdown of red blood cells, which leads to a decrease in the level of hemoglobin in the blood [24,25].

This study was conducted to know the hepatotoxic potential of iron overload on liver by measuring serum liver enzymes like ALT, AST, total bilirubin, total protein, and albumin concentrations. The results of the current study demonstrate a significant rise in serum ALT and AST concentrations in βTM group when compared with control group (table 2). This increase in enzyme concentration resulted from liberation of liver enzymes from broken hepatocytes to plasma as a result of toxic effect of iron to hepatocytes [26]. The significant positive association between serum ferritin and ALT, serum ferritin and AST concentrations in βTM group is due to the liver is the primary organ for iron storage in the body, thus that raised of serum ferritin level is associated to raised liver iron level and the opposite is true, this lead to more iron within the liver which cause more damage to hepatocytes and release of ALT and AST to blood, these results was consistent with Bashi and Fathi whose results showed significant increases in the mean serum levels of ALT and AST and ferritin as compared to the control [8]. Moreover, there is a significant negative association between serum hemoglobin and ALT, serum hemoglobin and AST concentrations in βTM group. This negative correlation resulted from increased hemolysis and ineffective erythropoiesis that led to decrease the hemoglobin and increase the iron i. e. increase the damage to hepatocytes and release of ALT and AST to blood. These results are consistent with study of Al-Moshary *et al*. who noticed the same reaction on the lipid function test on βTM [27].

Our study showed a significant increase of serum bilirubin in βTM group compared to controls group. The increase in serum bilirubin in βTM group is due to hemolysis of red blood cells that cause more hemoglobin converted to bilirubin i. e. increase the synthesis of bilirubin. These results agree with Mutlag results that showed significant correlation was found between the heterogeneity in the concentrations of liver enzymes (ALT, AST) and ferritin levels in patients with beta thalassemia [28]. The linear regression analysis and correlation coefficient statistical analysis show a significant relationship between serum ferritin and liver function. On the other hand, some research results showed correlation between AST and ALT levels and beta-thalassemia patients according to the correlation test [29].

Since the liver is the main site of protein synthesis and proteins have different functions, such as regulating osmotic pressure and transporting different molecules [30]. Abnormal protein and albumin levels are signs of liver dysfunction. There were significantly higher levels of albumin and total protein in βTM compared to the control group (Fig. 4). The current results are consistent with what was mentioned by the researcher Ayyash and Sirdah, who indicated that Serum ferritin that estimated in 40 cases showed highly mean level at 2402 ng/ml, and found highly significant correlation between serum ferritin and serum bilirubin beyond serum ferritin levels at (r=0.53), while the AST and ALT showed high statistically significant correlation with serum ferritin was found beyond at (r= + 0.62) [31].

CONCLUSION

It is concluded that a change in the concentration of liver enzymes (ALT, AST) or total bilirubin concentration in patients with βTM may be an indicator of hepatic impairment associated-iron overload.

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AUTHOR CONTRIBUTIONS

Conception and design of the study: GMH. Drafting the manuscript: MAA. Analysis and interpretation of data: NYH.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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