Hematological and Biochemical markers iron status in Thalassemic children receiving Multiple Blood transfusion

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Abstract

Background: liver disease has emerged as a major cause of mortality in patients with B- thalassemia major (TM). **Objective:** To assess the possible role of iron overload as a cause of liver dysfunction in thalassemic children receiving multiple blood transfusions and its correlation with serum aminotransferases.

Patients and methods: The present study was a one year descriptive cross-sectional study aimed to assess the possible role of iron overload as a cause of liver dysfunction in thalassemic children receiving multiple blood transfusions and its correlation with serum aminotransferases in children who attended to the Thalassemic Center at Hematological Department of Assiut University Children Hospital in the period from the first of January 2021 up to the end of December 2022. The study included 79 thalassemic children.

Results: In the current study showed that Statistically significant negative correlation was observed between the hemoglobin level & hematocrit (%) and the iron profile of the studied cases. Also, all studied iron profile showed statistically significant positive correlations with liver enzymes (ALT & AST levels). our study demonstrated that albumin synthesis decreased as the severity of iron overload increased. The coefficient correlation between iron profile and albumin was negative, unlike the transaminases. Another significant positive correlation was observed between iron profile of the studied cases and bilirubin level.

Conclusion: This study demonstrates that most children with thalassemia have iron overload. It was inferred that iron overload is a principle reason for raised liver enzymes (ALT, and AST), decreased albumin level, and increased bilirubin level.

Key wards: markers, Multiple Blood transfusion, thalassemia, serum aminotransferases.

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Introduction

Thalassemia is derived from the Greek words, Thalas, which means sea, and emia, which means blood, signifying that it is more common in the Mediterranean region [1]. Globally, among humans, thalassemia is the commonest single-gene disorder. It is defined as a group of inherited disorders characterized by decreased or absent beta globin chain synthesis, leading to a reduced level of hemoglobin (Hb) in the red blood cells. Specifically in developing countries, thalassemia is a huge health dilemma [2].

Beta Thalassemia is the most common chronic hemolytic anemia in Egypt (85.1%) with an estimated carrier rate of 9-10.2% [3].

Blood transfusion is the primary way of treating thalassemia; it allows the normal growth of the child as well as restrains abnormal erythropoiesis [4].

Iron-chelating agents should be used properly; otherwise, multiple blood transfusions can lead to iron overload. Yet, with no blood transfusion, the increase rate of erythropoiesis intensifies dietary iron absorption from the gut, leading to a severe form of iron overload [5].

The liver has the maximum capacity to store excess iron in the body, and various other organs, as well as the liver, are very susceptible to damage as a result of iron toxicity. The correlation between serum ferritin and hepatic iron concentration has been reported in multiple blood-transfused thalassemia patients [6, 7]

Patients and methods:

This study was a descriptive cross-sectional study. Hematology unit, Assiut University Children Hospital. In the period from the first of January 2021 up to the end of December 2022.

Inclusion criteria: Children and young adult aged 5–18 years. Thalassemic patients of both sex. Beta-thalassemia major patients diagnosed by clinical and laboratory investigations who undergoing multiple blood transfusion and attending to the Thalassemic Center at Hematology unit of Assiut University Children Hospital. Accept to participate in this study.

Exclusion criteria: Children aged less than 5 years. Children with acute illness as fever and infections. Children with hepatitis C or B infection.

Children or parents who refused to participate in this study.

Methodology:

Eligible children were subjected to the following preliminary evaluation: Complete history taking, Complete physical examination, and Laboratory investigations.

Operational design:

The researcher introduced himself to all participants included in this study and asked them to participate after illustrating the goal of the study in local languages.

All participants received comprehensive information regarding objectives and the expected benefits of the study. All ethical considerations were taken throughout the whole work.

Ethical committee:

The protocol of the study had been approved from Assiut Medical

Ethical Review Board (IRB No 17101530). All pediatric patients participate in this study had received their medical care free of charge. Patients refused to participate in the study did not affect quality of care they had received. An informed written consent from all participants or their parents in young aged children was taken and confidentiality of information was ensured all through the study.

Statistical analysis:

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 22. Data were statistically described in terms of mean ± standard deviation

(±SD), or median and range when not normally distributed, frequencies (number of cases) and relative frequencies (percentages) when appropriate. Correlation between various variables was done using Pearson correlation test. P-value set significant at 0.05 level

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Results

Table 1 Demographic data of the studied 79 thalassemic children who receiving multiple blood transfusions at Assiut University Children Hospital (AUCH)

Demographic data	**N=79**	
Age (years)		
• Mean ± SD	10.54 ± 3.67	
• Median (range)	10 (5 – 17)	
Sex		
• Male	42	(53.2)
• Female	37	(46.8)
Family history		
• No	35	(44.3)
• Yes	44	(55.7)
Consanguinity		
• No	40	(50.6)
• Yes	39	(49.4)

Quantitative data are presented as mean ± SD or median (range), qualitative data are presented as number (percentage).

The demographic data of the studied participants was summarized in **Table 1**. The mean age of the studied cases was 10.54 ± 3.67 years and ranged from 5 up to 17 years old. Out of 79 studied cases; 42 (53.2%) were males, and 37 (46.8) were females. Positive family history of thalassemic disease was documented in more than half (55.7%) of the studied cases, and positive consanguinity was reported in 39 cases (49.4%).

Table 2 Clinical data of the studied 79 thalassemic children who receiving multiple blood transfusions at AUCH

Clinical data	**N=79**
Onset of disease (months)	
• Mean ± SD	12.81 ± 7.76
• Median (range)	9 (6-79)
Age of start transfusion (months)	
• Mean ± SD	12.81 ± 7.76
• Median (range)	9 (6-79)
Weeks between every once blood transfusion	
• Mean ± SD	4.80 ± 1.45
• Median (range)	4 (2 – 10)
Splenectomy	
• No	56 (70.9)
• Yes	23 (29.1)
Weeks between every once blood transfusion before splenoectomy (weeks)	
• Mean ± SD	2.35 ± 0.65
• Median (range)	2 (1 – 4)
Weeks between every once blood transfusion after splenectomy (Weeks)	
• Mean ± SD	6.30 ± 1.69
• Median (range)	6 (4 – 10)

Quantitative data are presented as mean ± SD or median (range), qualitative data are presented as number (percentage).

Table 2 showed the mean age of disease onset was ranged from 6 up to 79 months. Twenty three cases (29.1%) underwent splenectomy, before splenectomy the studied participants were received one blood transfusion every two weeks on average and ranged from once blood transfusion every week up to once blood transfusion every four week, after splenectomy the studied participants were received one blood transfusion every six weeks on

average and ranged from once blood transfusion every four week up to once blood transfusion every ten week.

Table 3 Chelation therapy received by the studied 79 thalassemic children who receiving multiple blood transfusions at AUCH and its compliance

Chelation therapy	N	(%)
Receiving chelation therapy (n=79)		
• No	21	(26.6)
• Yes	58	(73.4)
Chelation therapy type (n=58)		
• Deferasirox (DFX)	52	(89.7)
• Deferiprone (DFP)	6	(10.3)
Chelation therapy compliance (n=58)		
• No	2	(3.4)
• Yes	56	(96.6)

Qualitative data are presented as number (percentage).

Among the studied 79 thalassemic children; only 58 cases (73.4%) received chelation therapy (89.7% receiving DFX, and 10.3% receiving DFP), and almost all those patients were complaint to their treatment, while two cases were not complaint to receiving DFX medication, as shown in **Table 3**.

Table 4 General and abdominal examination among the studied 79 thalassemic children who receiving multiple blood transfusions at AUCH

	N	(%)
General examination		
• Pallor	79	(100.0)
• Jaundice	62	(78.5)
• Thalassemic features	48	(60.8)
Abdominal examination Liver		
• Not palpable	3	(3.8)
• Palpable	76	(96.2)
Liver size (cm) below costal margin in midclavicular line , median (range)	6 (1 – 17)	
Spleen (n=56) • Not enlarged	6	(8.9)
• enlarged	50	(91.1)
Spleen size (cm) below costal margin in midclavicular line , median (range)	6 (2 – 20)	
Ultrasound examination Liver size		
• Normal	6	(7.6)
• Mild	36	(45.6)
• Moderate	32	(40.5)
• Huge	5	(6.3)
Spleen size		
• Normal	6	(10.7)
• Mild	26	(46.4)
• Moderate	16	(28.6)
• Huge	8	(14.3)

Table 4 showed that all studied cases were pallor, 62 cases (78.5%) have jaundice, and 48 cases (60.8%) have thalassemic facies. On abdominal examination we observed that liver was palpable in fast majority of the studied cases (96.2%), with median size of 6 cm (range 1 – 17 cm). Out of 56 cases without splenoectomy; spleen was enlarged in 50 cases (91.1%), with median size of 6 cm (range 2 – 20 cm). On ultrasound examination we observed mild hepatomegaly in 36 cases (45.6%), moderate hepatomegaly in 32 cases (40.5%), and huge hepatomegaly in five cases (6.3%), mild splenomegaly in 26 cases (46.4%), moderate splenomegaly in 16 cases (28.6%), and huge splenomegaly in 8 cases (14.3%).

Table 5 Complete blood picture before blood transfusion among the studied 79 thalassemic children who receiving multiple blood transfusions at AUCH

Complete blood picture	Mean ± SD	Median (range)
• WBCs (10^3/ul)	8.42 ± 2.18	8.4 (4.2 - 12.1)
• Hemoglobin (g/dl)	6.44 ± 1.35	6.3 (3.5 - 10.9)
• Platelets (10^3/ul)	356.80 ± 174.50	299 (107 – 879)
• RBCs (10^6/ul)	3.01 ± 0.91	2.7 (1.6 - 6.1)
• MCV (fl)	68.30 ± 7.85	69.9 (46.0 - 85.7)
• MCH (pg)	22.25 ± 3.91	22.7 (13.6 - 32.4)
• HCT (%)	20.40 ± 5.07	19.2 (9.5 - 37.4)
• RDW (%)	22.82 ± 6.53	22.2 (7.6 - 38.0)

WBCs: white blood cells; RBCs: red blood corpuscles; MCV: Mean corpuscular volume; MCH: mean corpuscular hemoglobin; HCT: hematocrit; RDW: red cell distribution width. Quantitative data are presented as mean ± SD or median (range).

The laboratory data of the studied thalassemic children was presented in **Table 5**.

Table 6 Iron profile among the studied 79 thalassemic children who receiving multiple blood transfusions at AUCH

Iron profile	Mean ± SD	Median (range)
• Serum ferritin (ng/dl)	**1754.20 ± 1001.28**	**1720.0 (98.0 - 3804.0)**
• Serum iron (ng/dl)	**208.04 ± 66.50**	**212.0 (85.0 - 337.0)**
• TIBC (mcg/dL)	**292.29 ± 27.55**	**290.0 (233.0 - 370.0)**
• Transferrin saturation (%)	**70.56% ± 21.13%**	**73.0% (32.0% - 100.0%)**

TIBC: Total iron binding capacity. Quantitative data are presented as mean ± SD or median (range).

The iron profile of the studied thalassemic children was presented in Table 6.

Table 7 Liver function among the studied 79 thalassemic children who receiving multiple blood transfusions at AUCH

Liver function	Mean ± SD	Median (range)
• ALT (U/L)	36.96 ± 26.75	29.0 (6.0 - 107.0)
• AST (U/L)	44.41 ± 28.28	37.0 (12.0 - 128.0)
• Serum albumin (mg/dl)	3.87 ± 0.47	3.9 (2.5 - 4.7)
• Total bilirubin (µmol/L)	1.61 ± 0.59	1.6 (0.50 - 3.0)
• Direct bilirubin (µmol/L)	0.43 ± 0.24	0.4 (0.1 - 1.0)
• Indirect bilirubin (µmol/L)	1.19 ± 0.39	1.2 (0.4 - 2.0)

ALT: Alanine transmarine; AST: Aspartate transaminase. Quantitative data are presented as mean ± SD or median (range).

The liver function of the studied thalassemic children was presented in Table 7.

Table 8 The correlation between the iron profile and CBC parameters among the studied 79 thalassemic children who receiving multiple blood transfusions at AUCH

CBC parameters		Serum ferritin	Serum iron	TIBC	TS
WBCs (10^3/ul)	**r**	0.042	0.104	0.044	0.091
	p value	0.710	0.363	0.702	0.423
Hemoglobin (g/dl)	**r**	-0.272	-0.251	0.114	-0.310
	p value	**0.015**	**0.026**	0.318	**0.005**
Platelets (10^3/ul)	**r**	0.081	0.110	0.025	0.094
	p value	0.480	0.335	0.829	0.410
RBCs (10^6/ul)	**r**	-0.175	-0.185	0.137	-0.238
	p value	0.123	0.102	0.229	**0.035**
MCV (fl)	**r**	-0.089	-0.033	-0.050	-0.030
	p value	0.436	0.774	0.663	0.790
MCH (pg)	**r**	-0.187	-0.117	0.056	-0.147
	p value	0.100	0.305	0.627	0.195
HCT (%)	**r**	-0.348	-0.350	0.036	-0.392
	p value	**0.002**	**0.002**	0.752	**0.000**
RDW (%)	**r**	0.042	-0.037	-0.190	-0.004
	p value	0.715	0.743	0.094	0.974

Significant negative correlations were observed between serum ferritin and hemoglobin level (r= -0.272, p=0.015), and hematocrit value (r= -0.348, p=0.002), similarly serum iron shows significant negative correlations with hemoglobin level (r= -0.251, p=0.026), and hematocrit value (r= -0.350, p=0.002), also transferrin saturation shows statistically significant negative correlations with hemoglobin level (r= -0.310, p=0.005), RBCs count (r= -0.238, p=0.035), and hematocrit value (r=

0.392, p<0.001). Other correlation were not significant (p>0.05, for all), as shown in **Table 8**

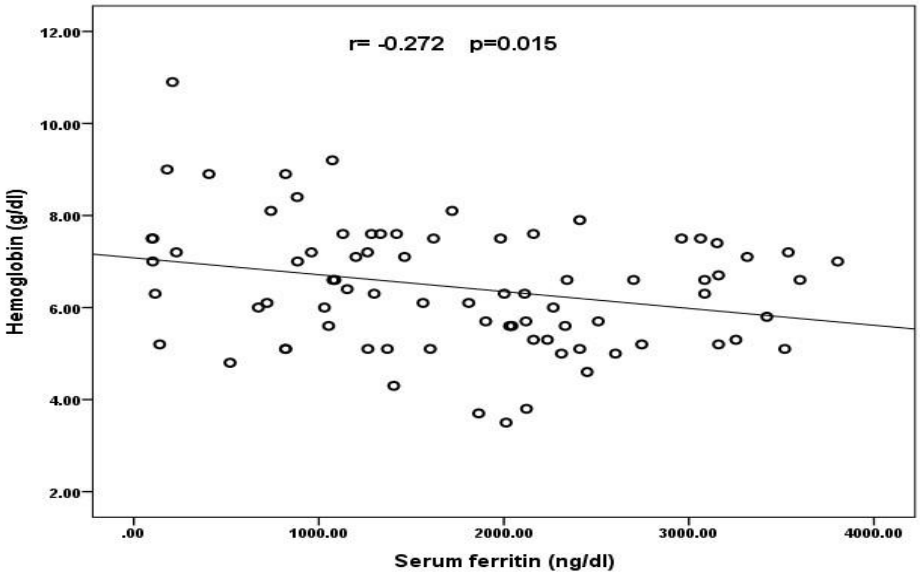

Figure 1 Scatter plot showing the correlation between serum ferritin, and hemoglobin level

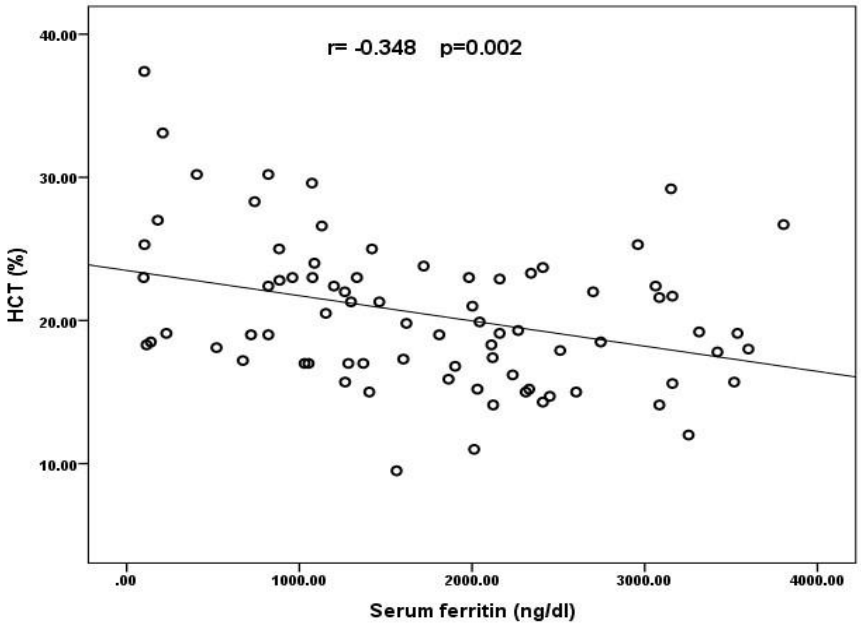

Figure 2 Scatter plot showing the correlation between serum ferritin, and hematocrit value

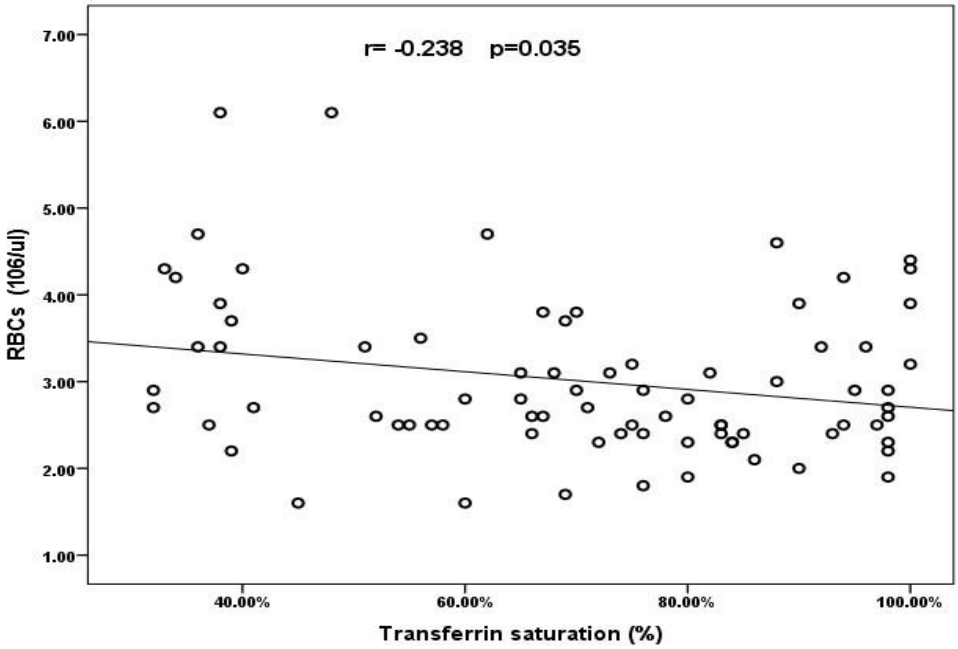

Figure 3 Scatter plot showing the correlation between TS and RBCs count.

Table 9 The correlation between the iron profile and liver function among the studied 79 thalassemic children who receiving multiple blood transfusions at AUCH

Liver function		Serum ferritin	Serum iron	TIBC	TS
ALT (U/L)	**r**	0.847	0.831	0.345	0.755
	p value	**<0.001**	**<0.001**	**0.002**	**<0.001**
AST (U/L)	**r**	0.815	0.803	0.372	0.721
	p value	**<0.001**	**<0.001**	**0.001**	**<0.001**
Serum albumin (mg/dl)	**r**	-0.752	-0.739	-0.335	-0.674
	p value	**<0.001**	**<0.001**	**0.003**	**<0.001**
Total bilirubin (µmol/L)	**r**	0.789	0.786	0.107	0.805
	p value	**<0.001**	**<0.001**	0.349	**<0.001**
Direct bilirubin (µmol/L)	**r**	0.709	0.737	0.230	0.707
	p value	**<0.001**	**<0.001**	**0.042**	**<0.001**
Indirect bilirubin (µmol/L)	**r**	0.759	0.738	0.024	0.783
	p value	**<0.001**	**<0.001**	0.835	**<0.001**

TIBC: Total iron binding capacity; TS: transferrin saturation; ALT: Alanine transmarine; AST: Aspartate transaminase. Significance defined by $p < 0.05$, r=correlation coefficient

Table9 showed all studied iron profile namely (Serum ferritin, iron, TIBC, and TS) show statistically significant positive correlations with ALT level (r=0.847, p<0.001; r=0.831, p<0.001; r=0.345, p=0.002; and r=0.755, p<0.001) respectively. Another significant positive correlation was observed between iron profile namely (Serum ferritin, iron, TIBC, and TS) and

AST level (r=0.815, p<0.001; r=0.803, p<0.001; r=0.372, p=0.001; and r=0.721, p<0.001) respectively.

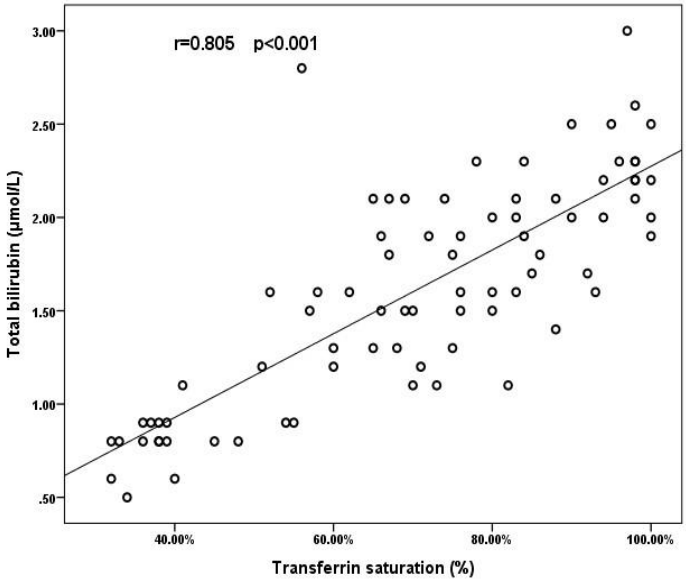

Figure 4 Scatter plot showing the correlation between TS and total bilirubin level

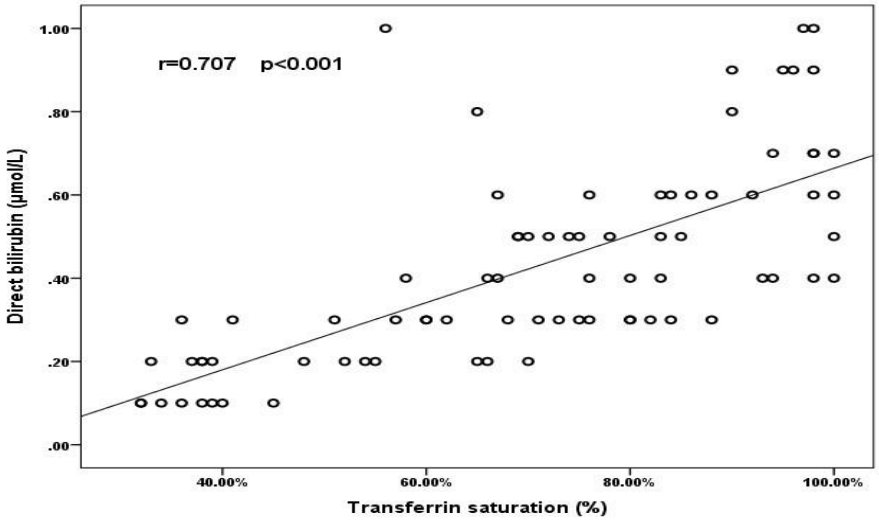

Figure 5 Scatter plot showing the correlation between TS and direct bilirubin level

Discussion

The study included 79 thalassemic children with a mean age of 10.54 ± 3.67 years and range (5-17 years old). Our results are in agreement with the previous Egyptian study of Elalfy et al., 2014 who reported that the mean age of children with B-TM was 11.80±4.78 years[8], Abdul-Zahra et al., 2016 study on 138 patients with b-TM reported that the mean age of the studied children was 9.36±4.48 years and ranged from 2 to 17 years old [9].

No gender predominance was observed among our studied cases with male: female ratio of 1.14: 1. Similar finding was reported by many previous studies [8,9].

There was high rate of consanguinity and positive family history among our studied cases as we observed that; consanguinity was positive in 49.5% of the parents with extended family history of thalassemia positive in 55.7%. In agreement with the current finding, Rudra et al. reported consanguineous marriage and positive family history of thalassemia in 72.5%, and 40.8% respectively [10]

Splenectomy is the usual recommendation when the annual transfusion requirement increases to or more than 200 to 220 mL RBCs/kg/year with a hematocrit value of 70% [11]. In the current study we observed that an increase in blood transfusion interval after splenoectomy.

Iron chelation therapy (ICT) is essential to prevent complications of iron overload in patients with transfusion-dependent thalassaemia[12] . In the current study only 58/79 cases (73.4%) received chelation therapy.

There are three main iron chelation agents including deferoxamine (DFO), deferiprone (DFP), and deferasirox (DFX)[13] . In the current study DFX was the mainly used chelation therapy received by 89.7% of our studied cases, and DFP was received by 10.3%.

However, there is currently no standard for how to best measure adherence to ICT, nor what level of adherence necessitates concern for poor outcomes, especially in pediatric patients. In the current study we only depend on the answer of the studied children or their parents about the compliance to their ICT, and we found that almost all patients were complaint to their treatment, while two cases were not complaint to their ICT (NB; both patients receiving DFX). This noncompliance to DFX may be contributed to the fact that DFX may be administered orally up to three times a day, also gastrointestinal distress and transaminitis are known side effects for DFX [13] .

The recent study of Abdelrahman et al. reported simmilar finding as they observed positive correlation between serum ferritin level and ALT & AST in the studied thalassemic patients. Consequently, the reason tends to be that the abnormal amounts of hepatic catalysts are potentially because of the hepatic damage caused by the iron overburden in thalassemia patients accepting a different blood transfusion [14]

Some analysts have depicted the proposed component of activity; however, the correct process is not clear as yet. Seng Suk et al. found liver functions to be three- to four-folds increased in β-thalassemia patients than normal individuals [15].

In addition; our study demonstrated that albumin synthesis decreased as the severity of iron overload increased. The coefficient correlation between iron profile and albumin was negative, unlike the transaminases. During albumin metabolism, only small portions of synthesized albumin are stored in liver, while the majority is released into the bloodstream. Any hepatocyte injury may cause decreased production of albumin [16]

Another significant positive correlation was observed between iron profile of the studied cases and bilirubin level. In line with the current study Al-Moshary et al. demonstrated significant positive correlation between serum ferritin and serum bilirubin level [17]

The liver is the earliest site of iron deposition in regularly transfused thalassemia patients and a common cause of morbidity. Iron overload occurs both in hepatocytes and reticuloendothelial cells. Free radical production is increased in patients with iron overload through the Fenton reaction. These free radicals accumulate in the liver, heart, and other organs cause extensive tissue damage and play havoc [18]. This highlights the need for regular monitoring of AST, ALT, albumin, and bilirubin levels in thalasemic children who receiving multiple blood transfusion as they may reflect the severity of liver iron overload in those patients, also further studies are needed to determine the real cause for this connection.

Conclusion

This study demonstrates that most children with thalassemia have iron overload. It was inferred that iron overload is a principle reason for raised liver enzymes (ALT, and AST), decreased albumin level, and increased bilirubin level.

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