Influence of blood transfusion rate on some clinical manifestations in β-thalassaemia major patients

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Background The commonest form of life-long treatment for individuals with β-thalassaemia major (TM) is blood transfusion; however, regular and multiple transfusion can result in iron overload as well as serious infections.
Objectives This study was designed to evaluate clinical manifestations in β-TM patients according to the blood transfusion rate.
Methods Forty-two patients of homozygous β-TM in the thalassaemia Division/Children's Teaching Hospital, Kerbela in the middle part of Iraq, during March–June 2015. All patients (17 males and 25 females) with age range 3–31 years were blood transfusion-dependent and on iron chelation therapy. They were divided into three groups according to the yearly average of blood transfusion received during the last 3 years; those who received less than 16 times/year (G1, n = 16), between 16 and 21 times/year (G2, n = 14) and more than 21 times/year (G3, n = 12). Several biochemical tests were carried out to estimate the serum level of ferritin, total serum bilirubin (TSB), GPT, GOT, alkaline phosphatase (ALP), albumin and total protein.
Results The results showed that half of patients were presented either with splenomegaly (28.6%) or splenectomy (21.4%), about one quarter of them (26.2%) were presented with hepatomegaly, more than half of patients (54.8%) were represented with HCV infection and the majority (97.6%) of patients have normal BMI. However, results of biochemical markers revealed that all of patients (100%) have albumin and total protein concentrations respectively, while all of them (100%) and the majority of them (76.2%) have elevated serum level more than the upper limit of normal reference of ferritin and TSB respectively. Additionally, liver enzymes levels (GPT, GOT and ALP) also recorded an elevation in about 31, 42.9 and 21.4% of thalassaemic patients. On the other hand, results demonstrated that increasing in the rate of blood transfusion revealed an association with the reduction in the frequency of patients with normal liver, spleen and negative HCV infection, and the three different groups of thalassaemic patients have significant differences in their age, BMI, rate of transfusion and total protein. However, the rest of biochemical tests (ferritin, TSB, GPT, GOT, ALP and Alb) revealed non-significant differences in their serum levels among the three groups of patients, but the percentage of patients that have elevated ALP were significantly different (0, 35.7 and 33.3%) (P = 0,029) among the G1, G2 and G3 respectively.
Conclusion Although blood transfusion is the commonest therapy to improve the life-span of β-thalassaemic patients worldwide, its rate should be reduced as less as possible to avoid the serious complications by searching for another criteria to indicate transfusion rather than haemoglobin concentration such as the antioxidant status of thalassaemic patients.
Keywords thalassaemia major, iron overload, liver enzymes, ferritin

Introduction

Thalassaemia major was firstly reported by Dr. Thomas Cooley in 1925 as classical, fatal and Mediterranean anaemia termed as Cooley's anaemia, now termed as β-thalassaemia major (TM).[1] β-Thalassaemia is found in Arabic countries especially those which are located on the Mediterranean, Saudi Arabia, Jordan, Syria and Yemen.[2] Survey in Iraq showed that β-thalassaemia trait is carried by 4.5–5% of the population,[3] and about 6–10% of the population have haemoglobinopathies of which thalassaemia is a major part,[4] specifically in the northern part of Iraq due to the high rate of consanguineous marriages in this region.[5,6] TM presents as a progressive anaemia during 6–24 months of age.[7] The anaemia is severe (Hb 2–7 g/dl) and has detrimental effects on most organ systems; in some patients, death would result without chronic blood transfusions.[7–9] According to the British Committee for Standards in Haematology, it has been recommended that the volume of transfusion is between 15 and 20 ml/kg depending on the pre-transfusion Hb and haematocrit of packed cells provided by the blood bank.[10,11] However, current practice for transfusion therapy in Arabian Gulf countries recommended that regular blood transfusions is usually administered for every 2–5 weeks to maintain the pre-transfusion Hb level above 9.5–10.5 g/dl.[12]

The present study was designed to evaluate the status of spleen, liver, body mass index, hepatitis C infection and some biochemical markers in β-TM patients according to the frequency of blood transfusion.

Material and Methods

Patients

Descriptive cross-sectional study was conducted in the thalassaemia Division/Children's Teaching Hospital, Kerbela in the middle part of Iraq, during March–June 2015. Forty-two patients of homozygous β-TM (17 males and 25 females) with age range of 3–31 years were included. All patients are blood transfusion-dependent (15–20 ml packed RBCs/kg, at 2–3 weeks interval) to maintain a pretransfusion haemoglobin concentration above 8 g/l, also all of them were on iron chelation therapy. From a written consent form, medical histories for all patients were recorded including: sex, age, blood groups, blood transfusion rate and complications in liver, spleen, and hepatitis C virus (HCV) infection. Patients were divided into three groups according to the yearly average of blood transfusion received during the last 3 years; those who received less

than 16 times/year (G1, n = 16), between 16 and 21 times/year (G2, n = 14), and more than 21 times/year (G3, n = 12).

Methods

The weight and height of all patients were measured to calculate body mass index (BMI). For adults (>20 years aged), a BMI of <18.5 is considered underweight, while a BMI >25 is considered overweight and above 30 is considered obese. For children and adolescents (2–20 years aged), a BMI that is less than the 5th percentile is considered underweight and above the 95th percentile is considered obese, while those with a BMI between the 85th and 95th percentile are considered to be overweight.[13] The blood samples were collected at morning, 2 weeks after previous transfusion. Three millilitres of venous blood were aspirated from cubital vein, and sera were obtained and stored at −20°C until to be used in the biochemical tests. A commercial reagents were used for the determination of serum ferritin level (Vidas® Ferritin, BioMerieux®, Lyon, France) based on an immunoenzymatic method with a final reading inflorescence (enzyme-linked fluorescent assay), according to procedures validated in our laboratory. Total serum bilirubin (TSB) concentration was determined by using a diagnostic kit (ACCENT-200, TSB, Poland) based on chemical oxidation, utilising vanadate as an oxidising agent and measuring the absorbance before and after the vanadate oxidation.[14] The concentrations of albumin (Alb) and total protein (TP) as well as three enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined according to the methods recommended by International Federation of Clinical Chemistry (IFCC) by using diagnostic kits from ACCENT-200, Poland.[15,16]

Statistical Analysis

Statistical analyses were carried out by using Vassar Stats web site for Statistical Computation.[17] Values were reported as the mean (M) ± standard error (SE) and one-way ANOVA test was used to compare study groups. Comparison of categorical data between the different groups was carried out with chi square test. All statistical tests were 2-tailed, and a *P* value of <0.05 was considered as statistically significant.

Results

The general characteristics extracted from the clinical profile of all patients under the permission of the thalassaemia centre were summarised in Table 1. The status of all patients in respect to their spleen, liver as well as HCV infection showed that half of patients (50%) have normal spleen, and the rest half of them were presented either with splenomegaly (28.6%) or splenectomy (21.4%). While liver status showed that about three quarter of patients (73.8%) have normal liver and about one quarter of them (26.2%) were presented with hepatomegaly. On the other hand, more than half of patients (54.8%) were represented with HCV infection positive.

The results of BMI values and other biochemical tests were demonstrated in Table 2. Since the normal range references of these parameters are variable according to sex and age of individual, therefore the frequency of normal and abnormal cases were calculated to reflect the actual impact of this disease on the health status of patients. This table revealed that the majority (97.6%) of patients and all of them (100%) have normal BMI, albumin and total protein concentrations respectively. In contrast, all of the patients (100%) and the majority of them (76.2%) have elevated serum level more than the upper limit of normal reference of ferritin and TSB respectively. Additionally, liver enzymes levels (GPT, GOT and ALP) also recorded an elevation in about 31, 42.9 and 21.4% of thalassaemic patients respectively. The gender occurrence and blood groups distribution, status of liver and spleen, as well as HCV infection among the different groups of TM patients (G1, G2 and G3) were summarised in Table 3. Although statistical analysis by using Chi-square test (χ^2) showed no significant

Table 1. **General characteristics of patients**

Character		Values
Gender (n) (%)	Female	25 (59.5%)
	Male	17 (40.5%)
	Total	42 (100%)
Age (years)	Min.	3
	Max.	31
	Average	18.3
Blood groups (n) (%)	A^+	15 (35.7%)
	B^+	9 (21.4%)
	AB^+	7 (16.7%)
	O^+	11 (26.2%)
Blood transfusion rate (times/year)	Min.	7.3
	Max.	28.4
	M ± SD	18.7 ± 4.8
Spleen	Normal	21 (50%)
	Splenomegaly	12 (28.6%)
	Splenectomy	9 (21.4%)
Liver	Normal	31 (73.8%)
	Hepatomegaly	11 (26.2%)
HCV infection	Positive	23 (54.8%)
	Negative	19 (45.2%)

Table 2. **The average values of BMI and other biochemical parameters and their abnormal frequency of overall patients**

Parameters	M ± SD	Cases (n) (%)	
		Normal	Abnormal
BMI (kg/m^2)	20.11 ± 3.72	41 (97.6%)	1 (2.4%) ↓
Ferritin (ng/ml)	3162.7 ± 2081.9	0 (0%)	42 (100%) ↑
TSB (mg/dl)	2.01 ± 1.35	10 (23.8%)	32 (76.2%) ↑
GPT (U/l)	37.6 ± 40.5	29 (69%)	13 (31%) ↑
GOT (U/l)	43.4 ± 33.8	24 (57.1%)	18 (42.9%) ↑
ALP (U/l)	148.1 ± 71.1	33 (78.6%)	9 (21.4%) ↑
Albumin (g/dl)	4.49 ± 0.23	42 (100%)	0 (0%)
TP (g/dl)	7.29 ± 0.62	42 (100%)	0 (0%)

(↓) less than the lower limit of normal reference
(↑) more than the upper limit of normal reference

Table 3. **Distribution of gender and blood groups among patients groups and their statistical significance**

Character		G1 (n = 16)	G2 (n = 14)	G3 (n = 12)	Chi-square P value
Gender	Male	8 (50%)	5 (35.7%)	4 (33.3%)	$\chi^2 = 0.988$ P = 0.610
	Female	8 (50%)	9 (64.3%)	8 (66.7%)	
Blood groups	A^+	5 (31.3%)	6 (42.9%)	4 (33.4%)	$\chi^2 = 8.126$ P = 0.228
	B^+	6 (37.5%)	2 (14.3%)	1 (8.3%)	
	AB^+	3 (18.7%)	3 (21.4%)	1 (8.3%)	
	O^+	2 (12.5%)	3 (21.4%)	6 (50%)	
Liver status	Normal	14 (87.5%)	10 (71.4%)	7 (58.3%)	$\chi^2 = 3.079$ P = 0.214
	Hepatomegaly	2 (12.5%)	4 (28.6%)	5 (41.7%)	
Spleen status	Normal	9 (56.2%)	7 (50%)	5 (41.7%)	$\chi^2 = 5.638$ P = 0.227
	Splenomegaly	4 (25%)	2 (14.3%)	6 (50%)	
	Splenectomy	3 (18.8%)	5 (35.7%)	1 (8.3%)	
HCV infection	Positive	6 (37.5%)	10 (71.4%)	7 (58.3%)	$\chi^2 = 3.556$ P = 0.168
	Negative	10 (62.5%)	4 (28.6%)	5 (41.7%)	

Table 4. **Analysis of variance of all measurements among different groups of thalassaemic patients**

Param eters (M ± SE)	Patients group			ANOVA P value
	G1(n = 16)	G2(n = 14)	G3 (n = 12)	
Age (years)	12.8 ± 1.7	22.9 ± 1.5	20.2 ± 1.08	<0.0001
BMI (kg/m²)	17.2 ± 0.73	21.8 ± 0.68	21.9 ± 1.07	0.00011
Transfusion rate (times/ year)	13.7 ± 0.54	19.4 ± 0.35	24.4 ± 0.69	<0.0001
Ferritin (ng/ml)	2951 ± 500	3368 ± 663	3204 ± 554	0.869
TSB (mg/dl)	2.03 ± 0.43	1.97 ± 0.36	2.05 ± 0.22	0.990
GPT (U/l)	28.3 ± 3.8	58 ± 16.9	26.4 ± 5.9	0.071
GOT (U/l)	40.2 ± 3.3	59 ± 14.2	29.4 ± 4.4	0.077
ALP (U/l)	170.6 ± 17.1	119.7 ± 12.6	151.3 ± 26.5	0.151
Alb (g/dl)	4.47 ± 0.06	4.44 ± 0.04	4.57 ± 0.07	0.366
TP (g/dl)	7 ± 0.16	7.6 ± 0.11	7.2 ± 0.18	0.046

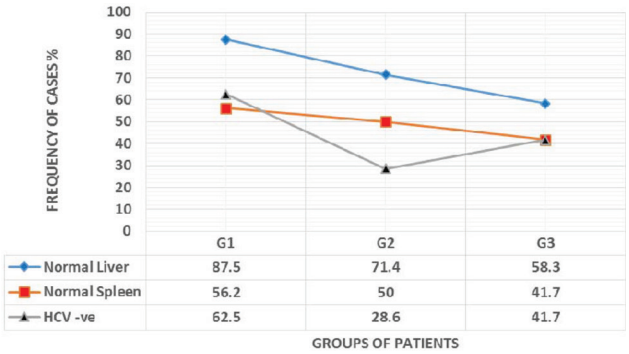

Fig. 1 **Frequency of cases with normal liver, spleen and absence of HCV infection among different groups of thalassaemic patients.**

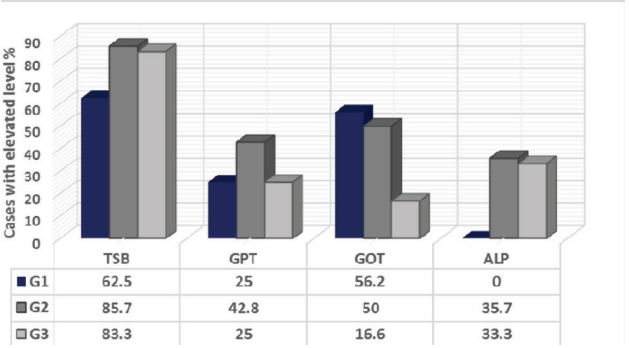

Fig. 2 **Frequency of cases with elevated serum level of biochemical markers among different groups of thalassaemic patients.**

association in all of these characters among the different groups, the cases of patients with normal liver, spleen and negative HCV infection were dramatically decreased as the transfusion rate increased (Fig. 1).

By using one-way analysis of variance, the significance of differences in age, BMI, transfusion rate and biochemical tests among the different groups of thalassaemic patients were demonstrated in Table 4. The results showed highly significant differences among the three groups in their age, BMI and transfusion rate; however, biochemical tests revealed non-significant differences (ferritin, TSB, GPT, GOT, ALP and Alb) except TP showed significant differences among different groups. The frequencies of cases that have elevated serum level of certain marker more than its upper limit of normal range were calculated. The results showed that 62.5, 85.7 and 83.3% of patients in G1, G2 and G3 respectively, have elevated TSB but without significant differences among them according to Chi-square test (Fig. 2). Similarly, those for GPT (25, 42.8 and 25%) and GOT (56.2, 50 and 16.6%) are non-significant. While those for ALP (0, 35.7 and 33.3%) revealed significant difference (P = 0.029) among the three groups of thalassaemic patients.

Discussion

This study was limited to one thalassaemia centre in Kerbela district/Iraq because it was difficult to recruit patients from other centres in other Iraqi cities, also β-TM seems to be more prevalent in Kerbela district which is mainly due to the high rate of consanguineous marriage among its population. Although an increasing number of TM patients are now treated with bone marrow transplantation, the majority of the patients still depend on regular transfusions.[18] All TM patients in this study were managed by blood transfusion approach at a rate of 18.7 times/year (Table 1) which is compatible with current practice for transfusion therapy in Arabian Gulf countries.[12] The patients of this study presented with several complications including splenomegaly or splenectomised (50% of all patients), hepatomegaly (26.2%) and HCV infection (54.8%) (Table 1). Several studies have been demonstrated that patients with β-TM may go through several complications as the transfusion-related infections like HBV, HCV and HIV.[19] Iron overload complications are also noticed that includes endocrinopathy, heart and liver diseases and chelation therapy complications.[20] Similarly, our results found that all patients (100%) were undergone iron overload with an average of ferritin level in about 3162.7 ng/ml, in addition to elevation in serum level of TSB, GPT, GOT and ALP in about 76.2, 31, 42.9 and 21.4% of total patients respectively, but those for albumin and total protein remain within their normal limits (Table 2). These results are compatible with those found by other researchers, some of them observed liver dysfunction in β-TM patients with a significant elevation in the activities of both aminotransferases enzymes (AST and ALT) in the sera of thalassaemic patient groups compared with control,[21,22] but no significant differences in the albumin levels of all patient groups compared to control was noticed.[22] However, other researchers found that iron over load and jaundice is common finding of thalassaemia and both serum ferritin and bilirubin parameters of iron over load and jaundice are correlated. But no statistical correlation was found between these two parameters.[23]

On the other hand, patients with haematological disorders such as TM have a high risk of exposure to HCV.[24] After the discovery of HCV in 1989, it was found to be the major cause of transfusion-associated hepatitis in the world.[25] Approximately, the prevalence of HCV in Iraq among TM patients was 66.6%,[26] which is nearly comparative to the prevalence among Saudi Arabia TM patients which was 70%.[27] As it is known, the complications of repeated blood transfusion were mainly developed from iron overload since every unit of blood contains ~200 mg of iron[28] that can be manifested by a high serum ferritin levels from transfusions[29] and may be exacerbated in some patients due to increased absorption of iron from the diet in response to ineffective erythropoiesis.[30] In contrary to these findings, our results found that the rate of blood transfusion does not revealed significant association in the distribution of sex, blood groups, status of liver and spleen as well as HCV infection among the three different groups (Table 3), although the cases of patients with normal liver, spleen and negative HCV infection were dramatically decreased as the transfusion rate increased (Fig. 1). Additionally, the most of biochemical markers (TSB, GPT, GOT, ALP and Alb) showed non-significant differences among the three groups (Table 4). These findings may be due to non-significant difference in the serum ferritin level in the different groups and all patients in these groups undergone iron overload, also the three different groups consist of patients with significantly different age and BMI (Table 4). Also our results revealed that all patients in G1 who received the lowest blood transfusion rate have normal level of ALP which is significantly differ from patients in G2 and G3 with further transfusion rate, whose ALP level were elevated in 37.5 and 33.3% of their patients (Fig. 2). This elevation of ALP in G2 and G3 may be due to two factors, the first one is hepatic toxicity due to serum ferritin as an index of iron overload which was high in G2 (3368 ± 663 ng/ml) and G3 (3204 ± 554 ng/ml) in comparison with that in G1 (2951 ± 500 ng/ml). Some researchers found that severe haemosiderosis and hepatic fibrosis were common in patients with TM despite the use of chelation therapy,[31] while others reported that patients with TM in Iraq are poorly managed of iron overload, though iron chelation is used, and clinical signs of iron overload appear in young thalassaemic patients due to poor control.[32] However, the second factor is the prevalence of HCV infection because the frequency of cases that have serum negative for HCV was dramatically decreased from 62.5% in G1 down to 41.7 and 28.6% in G3 and G2 respectively. The key problem with HCV infection is it's propensity to produce chronic liver disease, cirrhosis and hepatocellular carcinoma occurring after a number of years.[31] Since liver disease is a leading cause of death in patients with transfusion-dependent thalassaemia,[33] transfusion-associated hepatotropic infections, especially HCV infection, and hepatic siderosis can act either synergistically or independently in promoting chronic liver disease, and they may induce cellular damage through similar oxidative pathways.[34] Finally, this study demonstrated that the level of total protein was the only marker showed significant difference among the different groups, but its value was still within normal limits. This finding needs further investigation to search for protein–nature components in the blood of thalassaemic patients rather than those investigated in this study. ■

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