Effect of transfusion interval rate on clinical manifestations of patients with beta- thalassemia major and their correlation with some biochemical parameters

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Keywords*:* Beta- thalassemia major, Ferritin**,** Cortisol**,** Prolactin**,** BMI

Abstract

Background: Regular blood transfusions is the commonest form of life-long treatment for patients with beta-thalassemia major but multiple transfusions of blood causes iron overload and resulting in multiple progressive organ damage that later involved endocrine system, heart, and liver.

Objective: This study is aimed to evaluate some clinical manifestations in the adult patients with beta thalassemia major based on interval rate of the blood transfusion and also investigate correlation between clinical status of patients with some biochemical parameters including the level of ferritin, Hb, prolactin, and cortisol.

Methodology: Forty one Iraqi β -thalassemia major (TM) patients (19male and 22 female) are recruited from thalassemia center in Karbala at age of 14-24 years. The frequency of blood transfusion rate, blood groups, spleen status, and HCV infection are recorded from the profile of all patients. The weight and height of patients were measured to calculate body mass index (BMI), while the concentration of Hb, ferritin, cortisol, and prolactin are tested at morning just before blood transfusion session. Patients are grouped according to their transfusion interval rate (TIR) into two groups; short TIR group (< 3 weeks), and long TIR group (≥ 3 weeks).

Results: The results were non-significant in the distribution of gender and blood groups, spleen status, frequency of HCV infection, Hb, cortisol, and prolactin between the two groups, but age average, ferritin level and BMI of long TIR group are significantly lower than those of short TIR patients. On the other hand, long TIR patients revealed non-significant correlation between all parameters and clinical manifestations except for Hb content which is significantly decreased ($r = -0.451$, $P = 0.039$) in splenectomized patients. However, short TIR group showed that Hb content is significantly decreased in both splenectomized ($r = -0.560$, $P = 0.01$) and underweighted ($r = -0.477$, $P = 0.033$) TM patients.

Conclusion: We can concluded that the levels of cortisol and prolactin hormones are neither disturbed in TM patients nor correlated with interval rate of transfusion, but Hb level is the key factor that determine TIR and ferritin level leading to retardation in BMI which may be due to another endocrinopathy particularly those that are closely related with metabolic pathways such as growth hormone and thyroxin.

Introduction

The thalassemia is inherited disorder from the synthesis of haemoglobin caused by a change in the production rate of globin chain. Gene's mutations of globin of globin genes cause thalassemia. These mutations lead to weak synthesis of the β globin. The low rate of production of a certain globin chains (α, β, γ, and δ) hinders the synthesis of Hb and creates an imbalance with the others, which usually produced globin chains [1-3]. The β -thalassemia major is an autosomal recessive disorder, characterized by a reduction or absence of normal β -globin synthesis [1]. The beta-thalassemia is a serious genetic disorder which leads to a significant increase in both mortality and morbidity [4]. The beta-thalassemia major genes predominate in several ethnic groups spread across a wide geographic belt of the Mediterranean basin across the Middle East, , and Indian subcontinent [5].
Homogenous beta-thalassaemia will remain to be the one of the biggest health problem over the next few decades, particularly in developing countries [6]. Regular blood transfusions are the commonest form of life-long treatment for patients with β -thalassemia, but patients with multi-transfused thalassaemia major (TM) develop severe endocrine complications. Iron overload due to multiple transfusions is the main cause of such complications hence proper and effective iron chelation therapy is essential for the reduction of iron deposition on various endocrine glands. Iron accumulates in tissues with high levels of transferrin-receptor such as liver, heart and endocrine glands [7]. Therefore, the present study is aimed to evaluate some clinical manifestations in adult patients with beta thalassemia major based on the interval rate of blood transfusion and also to investigate the correlation between clinical status of patients with some biochemical parameters including the level of Hb, ferritin, cortisol, and prolactin.

Materials & Methods

Forty one Iraqi beta-thalassemia major (TM) patients (19 male and 22 female) were recruited from thalassemia Center /Children's Teaching Hospital, Kerbala in the middle part of Iraq, during June - October 2016 at age of 14-24 years. The frequency of blood transfusion rate, blood groups, spleen status, and HCV infection are recorded from the profile of all patients. Patients are grouped according to their transfusion interval rate (TIR) into two groups; short TIR group (< 3 weeks), and long TIR group (≥ 3 weeks). The body weights, by body balance, and height, by paper tape, of patients were measured to calculate body mass index (BIM) as weight in kilograms divided by height in meters squared [8]. Then, patients BMI were classified according WHO classification in (Table 1). At morning just before blood transfusion session, 5ml blood was collected from all patients. Hb level was determined by Gemmy hematocrit. Then, the rest of blood was collected in a dry sterilized test tube. The blood samples were centrifuged for 5 min at 3000 rpm to obtain serum which stored in -20C until be used for determination of ferritin level by an enzyme linked assay method using a kit supplied by Biomerieux (France), measured automatically with Minividas, Biomerieux (France), cortisol level according to Tosoh company and prolactin by two-site immune enzymometric assay using the ST AIA-PACK PRL, which is performed entirely in the AIA-PACK.

The results were computed as mean ± standard error for quantitative variables (age, weight, height, biochemical parameters tests and serum ferritin levels). Differences among groups were analyzed either one-way analysis of variance (ANOVA) or by Chi-square test for descriptive values. The correlation coefficient (r) was performed by Pearson correlation coefficient test. The P values of difference ≤ 0.01 were considered significant [9]..

Table1. BMI Classification

BMI(kg/m2)	Categories
<18.5	Underweight
18.5-24.9	Normal weight
25.0-29.9	Overweight
>30	Obese

Results

The basic clinical manifestations of TM patients are shown in table-2, in which only the age of TIR group (19 ± 0.70 years) is significantly higher (P = 0.005) than that of long TIR group (16.5± 0.48 years), while no significant differences found in the frequency of gender, blood groups, spleen and HCV infection status between the two groups.

Table-2: Clinical manifestations of TM patients according to their interval rate of blood transfusion

Character		**Short TIR (1) (n=20)**	**Long TIR (n=21)**	**Significance (2)**
Age (years)	Range	14-24	14-22	P = 0.005
	Mean ± SE	19 ± 0.70	16.5± 0.48	
Gender N (%)	♂	9 (45%)	10 (47.6%)	P = 0.866
	♀	11 (55%)	11 (52.4%)	
Blood Group N (%)	A	6 (30%)	5 (23.8%)	P = 0.496
	B	6 (30%)	3 (14.3%)	
	AB	1 (5%)	1 (4.8%)	
	O	7 (35%)	12 (57.1%)	
Spleen Status	Splenectomy (n, %)	13 (65%)	16 (76.2%)	P = 0.431
	Non-splenectomy (n, %)	7 (35%)	5 (23.8%)	
HCV infection status	Negative (n, %)	16 (80%)	19 (90.5%)	P = 0.342
	Positive (n, %)	4 (20%)	2 (9.5%)	

(1) Short Transfusion Interval Rate: One transfusion in < 3 weeks; Long TIR: One transfusion in ≥ 3 weeks

(2): P value of ANOVA for quantitative data; P value of Chi-square for qualitative data

The result of estimated parameters in both groups showed that TM patients with short interval of blood transfusion have a BMI value (20.7 ± 0.77 kg/m^2) and ferritin level (4024 ± 450 ng/ml) significantly higher (P = 0.022, P = 0.003 respectively) than those who received blood transfusion at long interval (18.2 ± 0.70 kg/m^2, 2394 ± 261 ng/ml respectively), however the rest parameters (Hb, cortisol, and prolactin) showed non-significant difference between the two groups of TM patients (Table-3).

Table-3: Estimation of Parameter's level in both groups of TM patients

Parameter Level	Normal Range	Sample value M±SE		Significance
		Short TIR (n=20)	Long TIR (n=21)	
BMI (kg/m^2)	≥ 20 years = 18.5-24.9 < 20 years = 5th-85th percentile	20.7 ± 0.77	18.2 ± 0.70	P = 0.022
Hb (g/dl)	♂ = 13.8-17.2 ♀ = 12.1-15.1	8.9 ± 0.21	9.1 ± 0.28	P = 0.568
Ferritin (ng/ml)	♂ = 18-270 ♀ = 18-160	4024 ± 450	2394 ± 261	P = 0.003
Cortisol (µg/dl)	7-28	10.9 ± 0.45	10.3 ± 0.90	P = 0.563
Prolactin (ng/ml)	♂ = 2-18 ♀ = 2-29	14.7 ± 2.5	11.0 ± 1.3	P = 0.205

When the BMI categories are calculated, the result showed that the frequency of underweight patients (47.6%) in long TIR group is significantly (P = 0.017) higher than that in short TIR group (10%), but in both groups obese category didn't recorded (Figure-1).

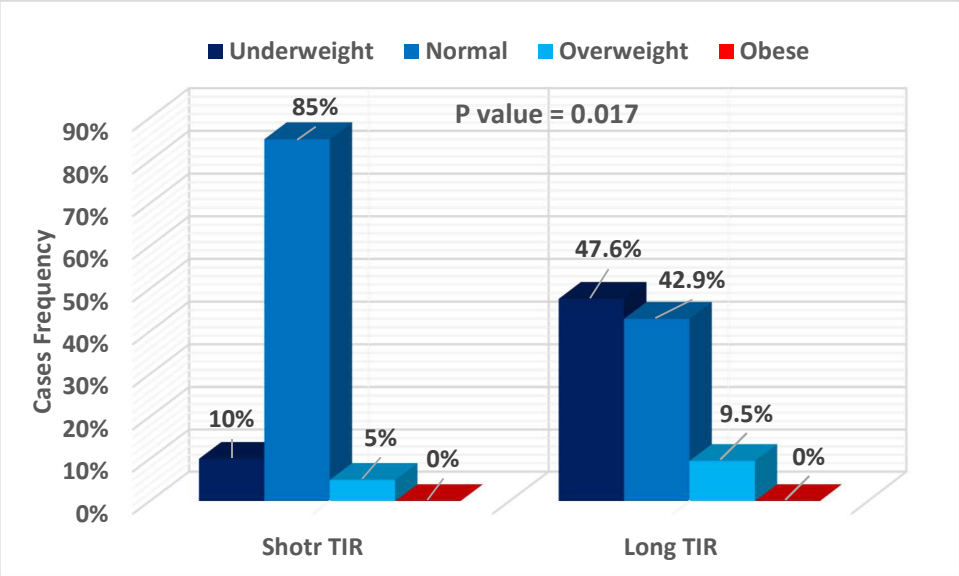

Figure-1: Frequency of BMI categories in TM patients according to interval rate of blood transfusion

By using Pearson's correlation test to evaluate the relationship between the tested parameters and clinical manifestations in both groups, TM patients with long TIR revealed non-significant correlation between all parameters and clinical manifestations except for Hb content which is significantly decreased (r = - 0.451, P = 0.039) in splenectomized patients (Table-4). However, short TIR group showed that Hb content is significantly decreased in both splenectomized (r = - 0.560, P = 0.01) and underweighted (r = - 0.477, P = 0.033) TM patients (Table-5).

Table-4: Correlation of clinical status versus parameters level in TM patients with long TIR.

Patient's status	Correlation (r) of Parameter Level versus			
	Hb	Ferritin	Cortisol	Prolactin
Age	r = 0.248 P = 0.277	r = - 0.147 P = 0.524	r = - 0.099 P = 0.698	r = - 0.034 P = 0.883
Splenectomy	r = 0.451 P = 0.039	r = 0.090 P = 0.698	r = - 0.342 P = 0.129	r = - 0.016 P = 0.965
Underweight BMI	r = - 0.079 P = 0.732	r = - 0.209 P = 0.384	r = 0.283 P = 0.212	r = 0.214 P = 0.350
HCV +ve	r = - 0.216 P = 0.347	r = - 0.218 P = 0.342	r = 0.304 P = 0.178	r = - 0.220 P = 0.337

Table-5: Correlation of clinical status versus parameters level in TM patients with short TIR.

Patient's status	Correlation (r) of Parameter Level versus			
	Hb	Ferritin	Cortisol	Prolactin
Age	r = - 0.133 P = 0.576	r = 0.166 P = 0.481	r = - 0.112 P = 0.638	r = 0.150 P = 0.527
Splenectomy	r = - 0.560 P = 0.010	r = 0.086 P = 0.715	r = 0.038 P = 0.870	r = 0.078 P = 0.743
Underweight BMI	r = - 0.477 P = 0.033	r = 0.172 P = 0.466	r = - 0.099 P = 0.677	r = 0.013 P = 0.956
HCV +ve	r = 0.058 P = 0.806	r = 0.388 P = 0.090	r = 0.144 P = 0.543	r = 0.199 P = 0.398

Discussion

Thalassemia described In 1925, by Lee and Cooley as a type of severe anaemia that associated with bone marrow changes and splenomegaly in children [10]. Transplantation of bone marrow is the only treatment, but regular blood transfusion is an available treatment for those patients [11]. Anaemia's sever complications could be reduced by regular and early blood transfusion therapy and prolong survival [12]. Blood transfusion in long term can lead to many complications such as alloimmunization against RBC, hemosiderosis, chronic viral infections [12], and iron overload which could explain higher level of ferritin in short TIR compared to the long TIR groups in this study. High iron level deposite in visceral organs like; liver, heart, and endocrine gland [13]. Major beta- thalassemia patients who receive regular blood transfusion are at risk of hepatitis such as; hepatitis B and C which is very common [14]. With time, splenomegaly occurs in thalassemia's patients that may worse anemia, causes thrombocytopenia, and neutropenia and can lead to thromboembolic. Splenectomy is the available treatment in this case [15].

In this study, the basic clinical manifestations of patients with TM are shown. There are higher frequencies of HIV and splenectomy in patients. While only age of short TIR group

significantly higher than that of long TIR group, no significant differences were found in the gender frequency, spleen, blood groups, and HCV infection status between the two groups. The highest frequency of Beta-thalassemia was in O blood group and the lowest was in AB group.

Mohssin *et al.*, who studied the frequency distribution of ABO blood group in thalassemia patients from Ibn-Baladi hospital in Bagdad/Iraq, reported that the incidence of thalassemia in O blood group is very common but in AB blood group is least common [16].

Mohammad *et al.*, also reported O^+ ve blood group to be common in the thalassemia patients in Iran [17]. While Saha *et al*., and Igbal *et al*., found that the B blood group is common among the donors [18, 19]. The RBC removal rate by spleen increase in patients with thalassemia; therefore RBCs, haemoglobin, haematocrit, and RBC levels are elevated after blood transfusion and splenectomy [20]. In present study, the elevation of HB level in different frequency of blood transfusion (short and long) didn't reach statistical significance, but there was significantly positive correlation between it and splenectomy. Many studies reported that children have thalassemia major suffered from many complications which include impaired immune function, growth retardation, and low body mass index [21-23]. It is well established that many parameters including age, gender, endocrine system status,

nutrition, and exercise that can influence the composition of the body [24] are weak in patients with thalassemia major in addition to hyperactivity of bone marrow, iron overload, and reduced bone mineral density [25, 26]. Fung *et al.*, reported that an increase underweight status among thalassemic patients, especially among adolescence and childhood [27]. This is possibly because of the presence of multiple endocrinopathies specially hypogonadism, under-nutrition [28-30], and also possibly the side effects of chelating therapy in long-term [31].

The results demonstrated, BMI was significantly higher in short TIR compared to long TIR. Underweight is significantly higher in long TIR compared to short TIR. Interestingly, underweight status has positive correlation with Hb in a long TIR. This result lead to suggest that the short TIR treat the reduction in Hb level faster and that will normalize the weight status in the donors. The classic knowledge was that in transfusion-dependent thalassemia patients, increased deposition of iron in pituitary gland has a cytotoxic effect, that leading main to hyper-gonadotrophic hypogonadism due to pituitary hypo-responsiveness to GnRH [32]. Hyper-gonadotrophic hypogonadism doesn't respond to chelation therapy that given late in the course of the disease [33]. Hyperprolactinemia appears to be not involved in the pathogenesis of hyper-gonadotrophic hypogonadism in thalassemia patients [32]. Levels of plasma prolactin were within the normal range in all female and male hypogonadal patients [34]. In this study, the prolactin levels were also within the normal range and there were no significantly differences between prolactin in the short and long TIR. The usual tests of adrenal function in the thalassemia patients showed little impairment of function but it is always working at maximum rate to produce the normal levels of circulating cortisol [35].

It was found that highly significant suppression of function in the physiological range with a normal reserve of function using the more physiological graded dose adrenal cortical stimulation test [36] . This could explain why the cortisol level was within the normal range

in the thalassemia patient in this study. There was also no significantly difference between cortisol levels in the short and long TIR.

Conclusion

From these findings, it can concluded that the levels of cortisol and prolactin hormones are neither disturbed in TM patients nor correlated with interval rate of transfusion, but Hb level is the key factor that determine TIR and ferritin level leading to retardation in BMI which may be due to another endocrinopathy particularly those that are closely related with metabolic pathways such as growth hormone and thyroxin.

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