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Research Article

EVALUATION SOME CONSEQUENCES OF THALASSEMIA MAJOR IN SPLENECTOMIZED AND NON-SPLENECTOMIZED IRAQI PATIENTS

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ABSTRACT

Objectives: Chronic blood transfusion therapy in thalassemia major patients caused excessive iron accumulation in different organs which was associated with high early fatalities. The aim of this study was to evaluate the body mass index (BMI), and serum levels of TSH, T4, T3, ferritin and bilirubin in splenectomized and non-splenectomized β-thalassemic patients.

Methods: Forty patients of homozygous β-thalassemia major (TM) and fifteen controls of matched age and gender were included in the study. Thalassemic patients were divided into splenectomized and non-splenectomized patients.From all patients medical history was taken and the needed data including sex, age, weight, and height were collected, and body mass index (BMI) was calculated. Blood samples were collected and serum levels of TSH, T4, T3, ferritin and bilirubin were determined.

Results: In comparison with healthy controls, the results showed significant ($p<0.05$) increased TSH (3.5 ± 1.7 μIU/ml), and decreased BMI (17.3 ± 4.8 kg/m^2) in the thalassemic patients, from them 20% with subclinical hypothyroid, 60% with underweight, 22.5% with hepatomegaly, and 10% have HBV and/or HCV infections. According to the analysis of differences between splenectomized and non-splenectomized thalassemic patients, most of investigated parameters (TSH, T4, T3, Ferritin, TSB, and blood transfusion rate) showed insignificant difference. However, BMI in splenectomized patients was significantly higher (18.2 ± 2.8 kg/m^2, 38.9% of them have underweight) than that of non-splenectomized patients (16.5 ± 2.2 kg/m^2, 77.3% of them have underweight). The blood transfusion rate revealed significant correlation ($r = + 0.503$, $p = 0.017$) only with the TSH level of non-splenectomized patients.

Conclusion: The present resultsneed further studies in different regions with more subjects to confirm the advantages and disadvantages of splenectomy, ferritin, and bilirubin in thalassemia major patients.

Keywords: Thalassemia major, Iron overload, Splenectomy.

INTRODUCTION

Thalassemia was first described by Cooley and Lee in 1925 as a group of children with similar clinical and hematological abnormalities [1]. The term thalassemia is derived from the Greek, thalassa (sea) and haima (blood) [2]. Beta-thalassemia major (TM) is a congenital hemolytic anemia caused by defects in β-globin chain synthesis and considered to be the most common autosomal single-gene disorder worldwide [3]. It can be found in more than 60 countries with a carrier (heterozygote) population of up to 150-200 million people or 4.5% of the world population, and at least 300,000 lethally affected homozygotes are born annually [4]. The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world and 1 in 10,000 people in the European Union [5]. Iraq is one of the countries in which 6-10% of the population have hemoglobinopathy of which thalassemia is a major part [6].

Treatment of thalassemia consists mainly of blood transfusion, chelation therapy and bone marrow transplantation. Blood transfusion and iron chelation therapy have improved the quality of life and life-span to an age of around 30 years [7], but frequent blood transfusions causes progressive iron overload, which is a major clinical complication of the treatment [8]. Iron overload can result in multiple progressive organ damage grouped together under a condition called hemosiderosis. Important complications of iron overload include growth retardation and delay of sexual maturation in children, and later involvement of the heart, liver, and endocrine system [5,9]. The frequency of hypothyroidism in Thalassemia patients ranges from 6 to 30% among different countries depending on chelation regimens [10]. Because splenectomy is often carried out to avoid complications associated with repeated transfusions and to minimize the need and frequency of blood transfusion, This study was conducted to evaluate the body mass index (BMI), thyroid functions, and serum levels of ferritin and bilirubin in splenectomized and non-splenectomized β-thalassemic patients.

MATERIALS AND METHODS

Patients

Control-based and cross-sectional studies were conducted in the thalassemia Division/ Children's Teaching Hospital, Karbalaa in the middle part of Iraq, during July - September 2012. Forty patients of homozygous β-thalassemia major (TM) (20 males, 20 females) with age range 11-30 year were included, and cases of β-thalassemia minor and intermedia were excluded from the study. All patients are blood transfusion-dependent (15ml packed RBCs/kg, at 2-3 weeks interval) to maintain a pre transfusion hemoglobin concentration above 8 g/l. All of the 40 patients studied were on iron chelation therapy. Desferrioxamine was given by subcutaneous infusion over 8-10 hours (20-50 mg/kg), generally 5-6 times weekly. According to control-based study fifteen controls (7 males and 8 females) of matched age and gender were also included in the study, while in cross-sectional study 40 patients were divided into two groups; G1(non-splenectomizedpatients, n=22), and G2 (splenectomized patients, n=18 p).

Methods

From all of the patients medical history was taken and complete physical examination was done and the needed data including sex, age, weight, and height were collected directly by the researchers after getting a written consent form. BMI (weight/height^2) at different ages was calculated. For adults (> 20years aged), a BMI of less than 18.5 is considered underweight, while a BMI greater than 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 [11]. All blood samples were collected at morning two weeks after previous transfusion. Three milliliters of venous blood were aspirated from cubital vein, and sera were obtained and stored at -20^o c until to be used in the biochemical tests. A commercial reagents were used for the determination of

serum ferritin, TSH, T3, and T4 levels (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) was determined by using bilirubin meter BR-501, Apel Company, Japan.

Statistical Analysis

Statistical analyses were carried out by using Vassar StatsWeb Site for Statistical Computation [12]. Values were reported as the mean (M) ± standard deviation (SD), and independent samples one-way Anova test was used to compare study groups. The associations between variables were assessed by using Pearson's correlation coefficient. Comparison of categorical data between the 2 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.

RESULT

Forty patients with thalassemia major (20 males + 20 females), and fifteen healthy individuals (control, 7 males + 8 females) are enrolled in the present study. The mean ± standard deviation for age, weight, height, BMI, and thyroid function tests are shown in table-1. The weight and BMI of thalassemic patients (40.6 ± 10.3 kg, 17.3 ± 4.8 kg/m^2 respectively) are significantly lower than those in control group (62.6 ± 15.3 kg, 25.1 ± 3.9 kg/m^2 respectively). However, TSH level in patient's group showed significant elevation (3.5 ± 1.7µIU/ml) in comparison with control group (2 ± 1.2µIU/ml).

The interpretation of BMI and thyroid function tests revealed that 24 patients (60%) are underweight, and 8 patients (20%) are subclinical hypothyroid, while control group recorded only one (7%) with subclinical hypothyroidism, and without any underweight status (0%) (Figure1).

Table 1: Analysis of variance between thalassemic patients and control groups

Parameters	Control (n=15)	Patients (N=40)	Normal Range	ANOVA test
Age(yr.)	18.8 ± 2.5 (13→30)	17.3 ± 4.8	…….	N.S
Weight(kg)	62.6 ± 15.3	40.6 ± 10.3	…….	P < 0.0001
Height(cm)	156 ± 10	152 ± 12	……	N.S
BMI(kg/m^2)	25.1 ± 3.9	17.3 ± 4.8	*5^{th}-85^{th} percentile(≤20yr)* *18.5-25 (>20yr)*	P < 0.0001
TSH (µIU/ml)	2 ± 1.2	3.5 ± 1.7	*0.25– 5 µIU/ml*	P = 0.004
T4(nmmol/l)	83.6 ± 14.6	77.8 ± 17.4	*60– 120 nmol/l*	N.S
T3(nmmol/l)	1.74 ± 0.52	1.65 ± 0.48	*0.92–2.3nmol/l*	N.S

Results expressed as mean ± standard deviation (SD).

NS: Not Significant.

Significantly different (P ≤ 0.01).

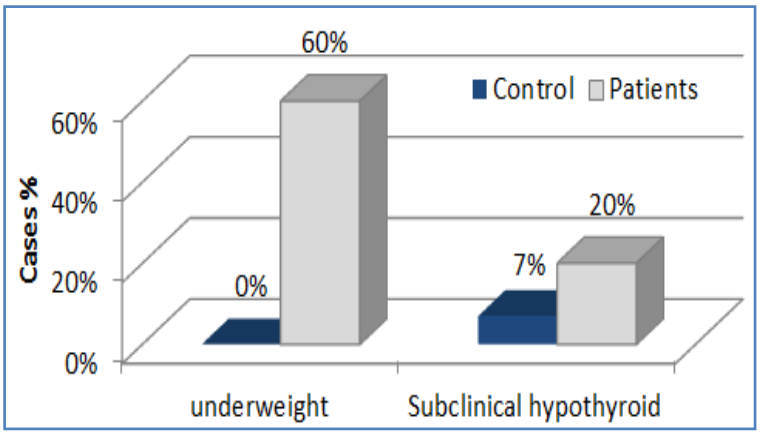

Fig. 1: Percentage of abnormal BMI & thyroid in patients and Control groups

Table 2: Analysis of variance between splenectomized and non splenectomized thalassemic patients

Parameters	Non splenectomized (n=22)	Splenectomized (n=18)	Normal Range	ANOVA test
Age(yr.)	15.5 ± 3	19.5 ± 5.6	……	P = 0.007
BMI(kg/m^2)	16.5 ± 2.2	18.2 ± 2.8	*5^{th}-85^{th} percentile(<20yr)* *18.5-25(>20yr)*	P = 0.041
TSH(µIU/ml)	3.8 ± 1.6	3.1 ± 1.7	*0.25 – 5 µIU/ml*	N.S
T4(nmol/l)	72.2 ± 20.6	78.6 ± 13	*60 – 120 nmol/l*	N.S
T3(nmol/l)	1.76 ± 0.49	1.51 ± 0.46	*0.92 – 2.3 nmol/l*	N.S
TSB (mg/dl)	1.62 ± 0.48	1.8 ± 1.06	*0.1 – 1 mg/dl*	N.S
Ferritin (ng/ml)	4894 ± 1125	4161 ± 1745	*30 – 350 µg/ml (male)* *20 – 250 µg/ml (female)*	N.S
Blood transfusion rate (times/year)	20.3 ± 3.7	21.1 ± 5.8	…….	N.S

- Results expressed as mean ± standard deviation (SD).
- NS: Not Significant.
- Significantly different (P < 0.05).

All of the tested parameters illustrated in the table-2 showed insignificant differences between splenectomized and non splenectomized patients except those related with age and BMI which are significantly higher in splenectomized patients (19.5 ± 5.6 years, 18.2 ± 2.8 kg/m^2 respectively) than non-splenectomized (15.5 ± 3 years, 16.5 ± 2.2 kg/m^2 respectively).

The Percentage of BMI (60% underweight), thyroid (20% subclinical hypothyroid), liver (22.5% hepatomegaly), and HBV / HCV infections (10% +ve) of patients in both groups were demonstrated in Table-3. According to Fisher Exact probability test, only the percentage of underweighted cases in the non-splenectomized patients is significantly higher than that in splenectomized patients (77.3%, 38.9% respectively). However, the rest manifestations showed insignificant differences between two groups.

The correlation of the frequency of blood transfusion versus BMI, TSH, TSB, and ferritin parameters in both thalassemic patient groups were statistically analyzed (Table-4). Frequency of blood transfusion revealed significant direct correlation with TSH (r = + 0.503, p = 0.017) only in the non splenectomized patients.

Table 3: Analysis of clinical picture differences between non-splenectomized and splenectomized patients

Groups	BMI status		Thyroid status		Liver status		HBV/HCV infection	
	Normal	Underweight	Euthyroid	Subclinical hypothyroid	Normal	Hepatomeg-aly	-ve	+ve
Non splenectomized	n=5	n=17	n=17	n=5	16	6	20	2
(n=22)	22.7%	77.3%	77.3%	22.7%	72.7%	27.3%	90.9%	9.1%
Splenectomized	n=11	n=7	n=15	n=3	15	3	16	2
(n=18)	61.1%	38.9%	83.3%	16.7%	83.3%	16.7%	88.9%	11.1%
Total	n=16	n=24	n=32	n=8	31	9	3	4
	40%	60%	80%	20%	77.5%	22.5%	90%	10%
Fisher Test	P = 0.023		N.S		N.S		N.S	

NS: Not Significant.

Significantly different (P < 0.05).

Table 4: Correlation of blood transfusion rate versus BMI, TSH, TSB, and ferritin in thalassemic patients

Groups	Non-splenectomy)				Splenectomy			
Parameters	BMI	TSH	TSB	TSB	BMI	TSH	TSB	Ferritin
Correlation (r)	N.S	+ 0.503	N.S	N.S	N.S	N.S	N.S	N.S
Significance (p)		0.017						

NS: Not Significant.

DISCUSSION

Our study investigated the distribution of height, weight, BMI and some of thyroid function tests (TSH, T4 and T3) in the blood of Iraqi patients with thalassemia major and healthy matched age and sex participants (control). It was found that the thalassemic patients had low weight and BMI (table-1), and nearly two third of them (60%) are underweight (figure-1) with significant differences from healthy participants. As decline in BMI was more obvious in thalassemic patients, it can be postulated that the developed endocrinopathies secondary to iron overload, and also possibly side effects of chelating therapy in long term are major contributing factors in producing underweight patients [13,14]. On the other hand our study demonstrated that 20% of thalassemic patients have subclinical hypothyroidism (figure-1) as a result of an elevation of thyroid stimulating hormone (TSH) more than 5 µIU/ml.

The frequency of hypothyroidism in thalassemia patients range from 6 to 30% among different countries depending on chelation regimens[9], therefore, lower prevalence was found in patients who had evidence of lower iron load as measured by ferritin levels [15].

Because splenectomy is one of the triad treatment of thalassemia major in addition to transfusion of red blood cells and chelation[16], this study also investigated the differences between splenectomized (n=18, 45%) and non-splenectomized (n=22, 55%) thalassemic patient. Our results found insignificant differences between two groups in majority of tested parameters; TSH, T4, T3, TSB, Ferritin, and blood transfusion rate (table-2). However, the average age of splenectomized patients (19.5 ± 5.6 years) was significantly higher than in non splenectomized patients. In the past, splenectomy was frequently performed inthalassemic patients because of hypersplenism and the new more intense transfusional regimens have decreased the need forsplenectomy. In addition it is now known that splenectomy increases the risk of sepsis and of thrombotic events [16]. Non-splenectomy group in the present study revealed low BMI (16.5 ± 2.2 kg/m^2) with high percentage of underweighted patients (77.3%) in comparison of those in splenectomized patients (18.2 ± 2.8 kg/m^2, 38.9% respectively). This result may be due to iron overload resulting in multiple progressive organ damage which includes growth retardation and delay of sexual maturation in children, and later involvement of the heart, liver, and endocrine system [5,8]. Although the concentrations of ferritin and total bilirubin recorded insignificant differences between splenectomized and non-splenectomized patients, they are highly elevated than their normal range (Table-2). Serum ferritin and serum bilirubin parameters are correlated in thalassemia major patients, but no statistical correlation was found between these two parameters[17]. The patients with β-thalassemia major usually suffer from iron overload as a consequence of recurrent transfusion and ineffective erythropoiesis. Iron has a catalytic role to produce powerful reactive oxidant species (ROS) and free radicals, which lead to oxidative damage [18-19]. Ferritin and bilirubin function as endogenous antioxidants and can result in increased level of total anti-oxidant status (TAS) in the patients with Beta-thalassemia major [20]. Because the frequency of blood transfusion plays a crucial role in thalassemia major complications, its statistical correlation with other tested parameters was analyzed which recorded significant direct correlation against TSH (r = + 0.503, p = 0.017) only in non-splenectomized patients (table-4). Although Blood transfusion and iron chelation therapy have improved the quality of life and life-span to an age of around 30 years, two factors may be contributed with fluctuation of thyroid status in thalassemic patients; age and the onset of chelation therapy [20]. It was found that thyroid impairment in some thalassemic patients was transient and their secretory capacity improved,while in the elderly patients with a late onset of chelation therapy, the detrimental effect of iron accumulation led to a permanent impairment of thyroid function suggesting that iron-induced toxicity is mainly time dependent[22]. Furthermore, patients with thalassemia major in Iraq are poorly managed of iron overload, though iron chelation is used, and clinical signs of iron overload appear in young thalassemicpatients due to poor control [23].

Finally, both groups of thalassemic patients revealed considerable percentage of subclinical hypothyroidism, hepatomegaly as well as HBV and/or HCV infections but without significant differences between them (table-3). Though transfusions may improve clinical symptoms for thalassaemia,this intervention is not curative and result in serious infections such as hepatitis B or C [24]. The interest in the clinical management of chronic liver diseases has been increasing, however,because of the high prevalence of viral infections in adulttransfusion-dependent thalassemia patients and the central role ofthe liver in regulating the iron metabolism [25].

CONCLUSION

This study found an increased in the frequency of underweight and subclinical hypothyroidism cases among thalassemic patients as a result of low BMI andhigh level of thyroid stimulating hormone (TSH) in comparison with the controls.Two significant differences were recorded between splenectomized and non-splenectomized thalassemic patients; firstly, enhancement of BMI that result in decreasing underweight frequency in splenectomized patients, and secondly the direct significant correlation of blood transfusion rate with TSH level in non-splenectomized patients which reflect the high incidence of subclinical hypothyroidism. Although increased serum levels of ferritin and bilirubin showed insignificant difference between splenectomized and non-splenectomized thalassemic patients, these endogenous antioxidantsmay protect thalassemic patients from consequences of oxidative damages resulted from iron overload. Further studies in different regions with more subjects are needed to confirm the results of this study.

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