Research article

Evaluation of leptin serum concentration in cases of blood transfusion dependent Beta thalassemia and its relationship with thyroid dysfunction

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ABSTRACT

Introduction and Aim: Thalassemia disorder is characterized by the body's inability to produce hemoglobin. This is an inherited disorder and arises due to defects in one or more globin chains. Thalassemia patients have been associated with endocrine dysfunction leading to toxic and deleterious effects. In the present study, we aimed to correlate leptin levels in Transfusion-dependent beta-thalassemia (TDT) patients to their thyroid hormonal levels and hematological parameters.

Materials and Methods: The study included 50 individuals (25 male and 25 female) aged 11-20 years with beta-thalassemia major and 20 healthy individuals (10 male, 10 females) aged 13-20 years as controls. All individuals included in the study were assessed for their BMI, complete blood count, serum ferritin and iron, thyroid function, leptin and ghrelin hormonal levels.

Results: This study showed a low BMI in patients as compared to healthy individuals. A high increase in TSH and ferritin was found in patients of both genders as compared to controls. T4 significantly decreased in males and females as compared to control. Significant reduction in leptin levels was observed in both male and female patients. A positive correlation was observed between leptin and TSH in males while a negative correlation between leptin and T4 was observed in females. A significant positive correlation was seen between leptin and T4 and between TSH and T4. BMI in males and female significant low compared to control.

Conclusion: Leptin probably plays an important role in thyroid dysfunction. Serum leptin, ferritin and thyroid hormonal levels in patients could be used as a guide in predicting hormonal modulation in major beta thalassemic patients**.**

Keywords: Leptin; thyroid function; beta-thalassemia.

INTRODUCTION

Thalassemia is an inherited genetic disorder that arises due to defects in one or more globin chains, affecting the body's ability to produce hemoglobin (1). Point mutations in the β-globin gene lead to a decrease (β^+) or lack (β^0) of production β-globin chain. β-Thalassemia can be classified according to the hematological and clinical severity in to three types namely, "β-thalassemia minor or trait", "β-thalassemia intermediate", and "β-thalassemia major"(2). Blood transfusions are considered as an essential method in treating thalassemia patients. However, studies have shown that repetitive transfusions could lead to an overload of iron in tissues and organs, hindering the normal functioning of glands and organs. Iron accumulation in the thyroid gland could lead to endocrinopathies like hypothyroidism and hypogonadism while in different tissues could be a cause to severe complications such as chronic liver disease, arrhythmias, infections, and heart failure (3).

Leptin, a polypeptide made-up of 146 -amino acids is produced by the adipocytes. The leptin hormone has several receptors and is responsible for energy homeostasis, regulated production of lipid, glucocorticoids, insulin and thyroid hormones in the body (4). In patients with β-thalassemia the adipocytes do not produce sufficient amounts of leptin and hence in these patients hormonal abnormalities is a major complication (5). Leptin is predominantly synthesized in the hypothalamus gland and therefore any disorder that effect on pituitary-hypothalamus possibly affects the thyroid-hypothalamus axis (6). Hospital based studies shown that hypothyroidism affects 6-30 percent of β-Thalassemia major (BTM) patients depending on the chelation techniques used (7). Hypothyroidism in turn can lead to various cardiovascular complications, such as a decrease in cardiac output due to a decrease in the blood which led to decreased the capacity of carrying oxygen and nutrition (8). Many hematological disorder associated with β-thalassemia are due to oxidative stress and generation of free radicles which are fundamental pathophysiological mechanisms leading to an

inflammatory cascade within RBCs and its destruction (9,10). A significant inverse relationship has shown to exist for body mass index (BMI) in children with β-thalassemia major (11). The main objective of this study was to evaluate and correlate the leptin level to hypothyroidism, hematological parameters and iron accumulation among male and female β-thalassemia major patients, in comparison to healthy individuals.

MATERIALS AND METHODS

This study included 70 individuals, 50 diagnosed as β-thalassemia major patients (25 males, 25 females) at the Al- Hussein Hospital, Iraq and 20 healthy individuals (10 males, 10 females) as controls. The mean age of male and female patients was 11.6±1.1 and 14.3±1.14 respectively. In the control group the mean age for male and female individuals was 16.4± 1.08 and 16.8±1.04 respectively. Blood (5ml) was withdrawn from each individual early morning and separated into two tubes. To one tube containing EDTA, 1ml of blood was added and tested for complete blood counts, while the remaining 4ml was transferred to another tube, for centrifugation (3000 rpm for 15 minutes) to separate out the serum. The serum obtained was stored at -20°C until further use.

Serum was tested for levels of leptin, iron, ferritin, and thyroid hormones. 40 µl of serum sample was used in measurng leptin by ELISA kit (BTLAB-China) method. Serum (250 µl) was measured for ferritin and thyroid hormonal (TSH, T3, T4) levels, using the ELISA kit (Biochem, Canada) as per the manufacturers instruction. Body mass index (BMI) of each individual was calculated by measuring their height (m) and weight (Kg) and using the formula $BMI = W_{Kg} / H^{m^2}$ (12).

Statistical analysis

Data obtained for samples was analyzed by using the SPSS version 24 software, employing the Pearson correlation analysis test for finding significance. The level of significance was observed at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$.

RESULTS

The results for blood parameters among male and female major thalassemia patients are shown in Table 1. A significant decrease in mean values for RBC, Hb, PCV, MCV and MCH parameters was observed in both male and female patients in comparison to controls. On the other hand an increase for WBC counts was observed in both males and female patients compared to controls. However, the increase was significant (p=0.05) only in males and not in female patients (Table 1). Similarly, an increase in PL parameter was observed in both genders, which was not significantly correlated. The MCHC values was observed to be lowered in male and female patients, with the decrease being statistically significant only in females (p=0.001) and not in males.

Tests for thyroid gland hormones revealed an increase in TSH hormone in patients of both genders which was highly significant ($p \leq 0.001$). On the other hand the T3 and T4 levels were found to decrease in comparison to levels in the control group, with the decrease being statistically significant ($p \leq 0.001$) only for T4 and not T3 hormonal levels (Table 2).

Serum was tested for iron and ferritin levels. Results demonstrated a high increase ($p \leq 0.001$) in ferritin among patients as compared to the control. The ferritin levels increased several folds with mean levels being as high as 3074.70± 466.48 and 4245.20±523.96 in males and females respectively. Body mass index (BMI) revealed highly significant ($p \leq 0.001$) decrease in these patients when compared to the healthy individuals (Table 3).

Table 1: Blood parameters of male and female thalassemia major patients and healthy (control) individuals

Parameters	Males				Females			
	Groups (n)	Mean	S.E.M.	P value	Groups (n)	Mean	S.E.M.	P value
RBC (10^6cell\µl)	Patient (20)	3.61	0.09	**0.001**	Patients (25)	3.59	0.12	**0.001**
	Control (10)	4.52	0.12		Control (10)	4.30	0.14	
WBC (10^3cell\µl)	Patient (20)	22.63	6.51	**0.05**	Patients (25)	15.22	3.39	0.13
	Control (10)	8.77	0.13		Control (10)	7.61	0.52	
Hb g\dl	Patient (20)	8.10	0.25	**0.001**	Patients (25)	8.28	0.23	**0.001**
	Control(10)	17.26	0.31		Control (10)	16.15	0.50	
PL (10^4cell\µl)	Patient (20)	405.85	43.49	0.32	Patients (25)	441.15	41.25	0.09
	Control(10)	342.30	17.92		Control (10)	338.40	11.09	
PCV%	Patient (20)	23.94	0.79	**0.001**	Patients (25)	24.76	0.69	**0.001**
	Control (10)	51.90	0.97		Control (10)	48.70	1.51	
MCV fl	Patient (20)	72.55	3.64	**0.01**	Patients (25)	75.14	1.50	**0.03**
	Control (10)	88.30	1.69		Control (10)	84.85	5.17	
MCH pg	Patient (20)	23.53	0.52	**0.001**	Patients (25)	23.57	0.55	**0.001**
	Control (10)	39.20	6.21		Control (10)	36.14	4.96	
MCHC g/dl	Patient (20)	30.30	1.44	0.11	Patients (25)	30.82	0.38	**0.001**
	Control (10)	63.47	29.06		Control (10)	32.76	0.40	

RBC: Red blood cells; WBC: White blood cells; Hb: hemoglobin; PL: platelets; PCV: packed cell volume MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration

Table 2: Thyroid hormonal levels in male and female thalassemia major patients and in control group

Parameters	Males				Females			
	Groups	**Mean**	**S. E.M.**	**P value**	**Groups**	**Mean**	**S. E.M.**	**P value**
TSH (mU/L)	Patient (20)	6.73	0.28	**0.001**	Patients (25)	6.47	0.30	**0.001**
	Control (10)	1.91	0.36		Control (10)	1.62	0.44	
T3 (ng/dl)	Patient (20)	1.79	0.17	0.41	Patients (25)	1.48	0.14	0.57
	Control (10)	2.28	0.77		Control (10)	1.30	0.37	
T4 (µg/dl)	Patient (20)	56.29	4.52	**0.001**	Patients (25)	62.16	4.21	**0.001**
	Control (10)	123.60	15.30		Control (10)	106.59	15.70	

Table 3: Iron, ferritin, BMI in the thalassemia major patients (males and females) and control group

Parameters	Males				Females			
	Groups (n)	**Mean**	**S. E.M.**	**P value**	**Groups (n)**	**Mean**	**S.E.M.**	**P value**
Iron µg\dl	Patient (20)	121.94	27.01	**0.58**	Patients (25)	96.48	25.91	**0.27**
	Control (10)	99.52	17.30		Control (10)	54.85	7.23	
Ferritin ng\ml	Patient (20)	3074.70	466.58	**0.001**	Patients (25)	4245.20	523.96	**0.001**
	Control (10)	87.20	13.68		Control (10)	77.30	12.91	
BMI kg\m^2	Patient (20)	20.66	1.15	**0.001**	Patients (25)	26.10	1.02	**0.001**
	Control (10)	31.00	1.83		Control (10)	31.65	1.10	

n= number of samples; S.E.M.= Standard error mean

The result of present data found a decrease (P≤0.0001) in leptin levels in male and female patients (1.21± 0.10), (1.51 ±0.14) as compared to the control group (8.36±0.63) (9.77±0.47) respectively (Table 4). A positive significant (P≤0.05) correlation was observed for leptin and TSH (r=0.51) levels, while a negative significant correlation (P≤0.05) for leptin and T4 hormone (-0.61) was seen among male patients (Table 5). Similarly, in females a positive correlation (P≤0.05) between Leptin and TSH (0.540) and a negative significant (P≤0.05) correlation between Leptin and T4 (-0.515) was observed. Correlation analysis for leptin and ferritin levels showed the two parameters to be negatively correlated and the association being highly significant (P≤0.01) in both male and female patients (Table 6). BMI was also to be significantly correlated to ferritin levels both patients of both genders (Table 6).

Table 4: Comparison of leptin levels in major thalassemia patients and control group

Parameter	Gender	Groups (n)	Mean	S.E.M.	t-test	P value
Leptin (ng\ml)	Males	Patients (25)	1.21	0.10	-15.63	0.001
		Control (10)	8.36	0.63		
	Females	Patients (25)	1.51	0.14	-21.50	0.001
		Control (10)	9.77	0.47		

n= number of samples; S.E.M.= Standard error mean

Table 5: Correlation of leptin levels to thyroid hormones among major thalassemia patients

Patients	Parameter	TSH	T3	T4	Leptin
Males	T3	0.269			
	T4	0.151	0.096		
	Leptin	0.511*	-0.218	-0.61**	
Females	T3	-0.236			
	T4	-0.515*	0.368		
	Leptin	0.540*	-0.259	0.172	

Significant difference at P≤0.05* and P≤0.01**

Table 6: Correlation between leptin with ferritin and BMI in thalassemia major patients

Patients	Parameters	Leptin	Ferritin
Males	Ferritin	-0.627**	
	BMI	0.640**	-0.524**
Females	Ferritin	-0.526*	
	BMI	0.541**	-0.413*

Significant difference at P≤0.05* and P≤0.01**

DISCUSSION

Thalassemia is a hereditary condition that affects the production of red blood cells (12). In the present investigation we observed that the physiological blood parameters such as RBC, Hb, Pl PCV, MCV and MCH significantly decreased in major thalassemia patients which is in agreement to a previous study by Alathari and Mahdi, 2019 (13) who studied these parameters in females suffering from major thalassemia disorder. The blood parameters Hb, MCV and MCH have also been reported to decrease significantly in β-thalassemia patients (14) and this decrease in RBCs has been related to defective hemoglobin chains leading to defects in RBC formation by hematopoiesis stem cell in the bone marrow (15). The low levels of Hb and RBC (anemia) exhibited by β-thalassemia patients is as a consequence of short RBC life span, hemolysis and ineffective erythropoiesis (15, 16). Increased concentration of ferritin observed in this study probably is related to repeated blood transfusions, excess iron accumulation and an increase destruction to RBCs (17). In the present study hypothyroidism was seen in all patients with beta thalassemia major which is in agreement with that reported by previous studies (7,9). The hypothyroidism observed was found correlated to the increased ferritin levels, which is in agreement to the study by Fung *et al.,* (18) who also found high increase in ferritin level with hypothyroid patients and attributed this to the iron overload which causes cellular damage and to the production of thyroid hormone.

In this study the BMI of patients exhibited an inverse relationship to leptin and ferritin serum concentrations. This observation is in accordance to an earlier report by Shahramian *et al.,* (4). The decrease in BMI, a factor used in growth assessment, could be related to several reasons such as endocrinopathies especially for hypothyroidism and hypogonadism, nutritional factors, iron overload effecting the production of growth hormones (13). Leptin produced by fat cell of hypo-thalamus is known to regulate the production of several hormones such as thyroxine and reproductive hormones (5). Present result showed significantly high decrease in leptin levels and the decrease being correlated to TSH levels. This correlation is in agreement to an earlier study, which reported hypothyroidism and low leptin levels to be related (5) attributing it to defect in the signaling pathway of hypothalamus and pituitary gland, leading to lowered fat mass which in turn decreases leptin production in white adipose tissue (5). The result in this study was in disagreement with an earlier study (19) which reported no significant correlation to exist between leptin, TSH and T3. Yet in another study leptin was found to play an inverse role in thyroid functioning by signaling the neuroendocrine hypothalamic-pituitary axis in transforming the hormone T4 to T3 hormone thereby controlling TSH secretion (20). Results for leptin levels in males and females in this study showed the levels to significantly decrease in these patients irrespective of the gender. This contradicts to an earlier finding which reported that leptin levels to be significantly lowered in major beta thalassemic men than in major beta thalassemic women (21). Decreased leptin production by major beta thalassemic patients as seen in this study could lead to deleterious effects such as ferritin overload leading to severe endocrinopathies and toxic effects in these patients (4).

CONCLUSION

There was a significant negative correlation of leptin levels with TSH, ferritin and BMI. Serum leptin levels in patients can be used to predict hormonal modulation in major beta thalassemic patients. More study is necessary to understand the physiological and biochemical alterations in these patients that impact their levels of serum leptin.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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