

# Evaluation of Serum Leptin and Ferritin Levels in Children with Major Beta-Thalassemia

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## Abstract:

**Background:** Beta-thalassemia major regarded as one of the major health problems in endemic regions as the Mediterranean basin, part of North and West Africa and South East Asia. Endocrine complication occur in major beta thalassemia due to excessive iron accumulation in endocrine glands that result from frequent blood transfusion and chelation therapy that caused dysfunction in their hypothalamic-pituitary axis. The aim of the study is to determine the level of serum Leptin and to determine the relation of leptin level with ferritin level in children with beta-thalassemia major.

**Patients & Methods:** A cross sectional study was carried out in Kirkuk City from January 2018 to March 2018 on 60 patients (30 female and 30 male) with beta thalassemia major and 30 age and sex matched healthy control (17 female and 13 male). Serum Leptin and ferritin levels were measured for both group by using commercial diagnostic kit.

**Results:** The result of the present study showed that serum level of leptin hormone in children with beta thalassemia major less than control subjects but not statistically significant. While the differences in the serum ferritin level was significantly higher in children with beta –thalassemia major as compare with control subjects.

**Conclusion:** Children with beta thalassemia major had a low level of leptin hormone than control subjects but not significant of same age and gender. Children with beta thalassemia major had a higher serum ferritin level than control subjects. Children with beta thalassemia major had a higher leptin level in female than male.

**Keywords:** Serum Leptin, Serum Ferritin, Beta-Thalassemia major

## Introduction:

Thalassemia is one of the most common inherited blood disorder characterized by decrease or absent synthesis of globin chains, there are two types of thalassemia, alpha and beta-thalassemia<sup>(1)</sup>. Beta-thalassemia results from a defect in production of beta globin chain due to mutations in the HBB gene on chromosome 11, inherited in an autosomal recessive fashion. Beta-thalassemia major is serious blood disorder since affected individual are unable to made healthy red blood cell and dependent on blood transfusion for survival. Children with

beta thalassemia major appear healthy at birth, but during the first year or two of life become severely anemic and depends on frequent blood transfusions throughout the life<sup>(2,3)</sup>. Endocrine complication in major beta thalassemia occur due to excessive iron accumulation in endocrine glands that result from frequent blood transfusion and chelation therapy that caused dysfunction in their hypothalamic-pituitary axis, leptin, an adipocyte derived hormone, primarily acts in hypothalamus, toxic effect of iron overload lead to decrease leptin

production, results in persistent immaturity of its hypothalamic-pituitary function<sup>(4)</sup>. Leptin is a polypeptide hormone with 146 amino acid, this hormone produced by lipocyte in many adipose tissue, which is widely regulate food intake, energy balance, inflammation, lipid metabolism and blood pressure<sup>(5)</sup>. Leptin can avoid cell apoptosis, especially muscle cell, a cardiac muscle cell line create a classified layer on the fractured parts of fat in a single layer and play a protective role against death cardiac cells therefore decrease the risk of cardiac infraction<sup>(6)</sup>. Leptin has important roles in regulating the blood pressure, it regulating hypertension as a risk factor for cardiac disease and infarction, cardiac problem is the most important problem in patient with thalassemia and the mean cause of death<sup>(7)</sup>.

Ferritin is a intracellular globular protein that found in most tissue consist of 24 subunit it stores iron, but small amount of ferritin secreted in to the serum, ferritin function is iron carrier and reflect iron storage in the body<sup>(8)</sup>. Frequent blood transfusion and chronic hemolysis lead to iron overload in thalassemia patients. Serum ferritin level is common test to evaluate iron overload in major beta-thalassemia<sup>(9)</sup>.

### **Patients and Methods:**

A cross sectional study was carried out in Kirkuk city from January to March 2018. The number of children with beta thalassemia major understudy were 60 (30 female and 30 male) whose ages were between ranging from 2-10 years of age, these patients admitted to thalassemia center in Azadi teaching Hospital and children hospital, and 30 (17 female and 13 male) healthy control ranging from 2-10 years old. Blood

samples (5ml) were collected by vein puncture using Vacutainer tubes from each patient enrolled in this study. Blood samples were placed into two sterile test tubes, one of them 2ml of blood was put in test tube containing anticoagulant EDTA and used for assessment of Hb and PCV tests. The second part of sample was 3ml placed in plane tubes left for 30 minutes at 37 °C then were centrifuged for 15 minutes and labeled for measurement of Serum Leptin by using Enzyme linked Immune assay technique (ELISA) and Serum Ferritin by using Enzyme linked fluorescent assay technique (ELFA). The comparison of categorical data were compared by using t-test, P-value < 0.05 was considered statistically significant.

### **Results:**

Regarding Age and body mass index (BMI) of female thalassemic patients , there was no significant difference between the Age of female patients group ( $7.39 \pm 2.22$  years) and female control group ( $6.58 \pm 2.25$  years ), ( $P > 0.05$ ), while the difference in the BMI of female patients group ( $21.8 \pm 1.64$  Kg/m<sup>2</sup> ) less than control group ( $23.8 \pm 1.12$  Kg/m<sup>2</sup> ), ( $P > 0.05$ ), but not significant, as shown in table (1).

Moreover , there is no significant difference between the Age of male patients group ( $6.74 \pm 2.51$  years) and male control group ( $5.46 \pm 2.70$  years ), ( $P > 0.05$ ) . There was a difference in the BMI of male patients group (  $17.01 \pm 1.98$  Kg/m<sup>2</sup> ) less than control group (  $19.7 \pm 2.09$  Kg/m<sup>2</sup> ), ( $P > 0.05$ ), but not significant, as shown in table (2).

Moreover, the serum leptin hormone in patients group ( $191.86 \pm 127.01$ pg/ml) less than control group ( $234.97 \pm 154.62$  pg/ml), ( $P > 0.05$ ) but not significant, as

shown in table (3). According to gender the serum leptin level in female patients group ( $243.40 \pm 141.14$  pg/ml) is less than female control group ( $264.53 \pm 166.04$  pg/ml) but not significant, ( $P > 0.05$ ), as shown in table (3). Also, there was a difference between the level of leptin hormone in male patients group ( $140.32 \pm 86.03$  pg/ml) less than control group ( $196.31 \pm 134.75$  pg/ml) but not significant, ( $P > 0.05$ ), as shown in table (3).

The data analysis indicate a statistically significant increase in serum leptin level in female ( $243.40 \pm 141.14$  ng/ml) in compare with male ( $140.32 \pm 86.03$  ng/ml), ( $P < 0.001$ ), in children with beta thalassemia major.

Regarding Ferritin level, there was a high significant difference between the

level of Ferritin in patients group ( $922.16 \pm 388.85$  ng/ml) and control group ( $35.01 \pm 23.30$  ng/ml), ( $P < 0.001$ ), as shown in table (3). Also, there was a high significant decrease in the level of hemoglobin (Hb) in patients group ( $8.27 \pm 1.15$  g/dl) than control group ( $12.85 \pm 0.53$  g/dl), ( $P < 0.001$ ), as shown in table (3).

Regarding Packed Cell Volume, there was a high significant decrease in the level of PCV in patients group ( $24.37 \pm 3.67$  %) than control group ( $38.70 \pm 1.37$  %), ( $P < 0.001$ ), as shown in table(3). Our Study showed negative relationship between serum leptin and serum ferritin level, (Regression ( $R^2$ ) = 0.0042 and ( $R$ ) = 0.064) but not significant, as shown in figure (1).

**Table (1):** The Mean $\pm$  SD of Age, BMI and Serum Leptin of Female Patients and Control Groups.

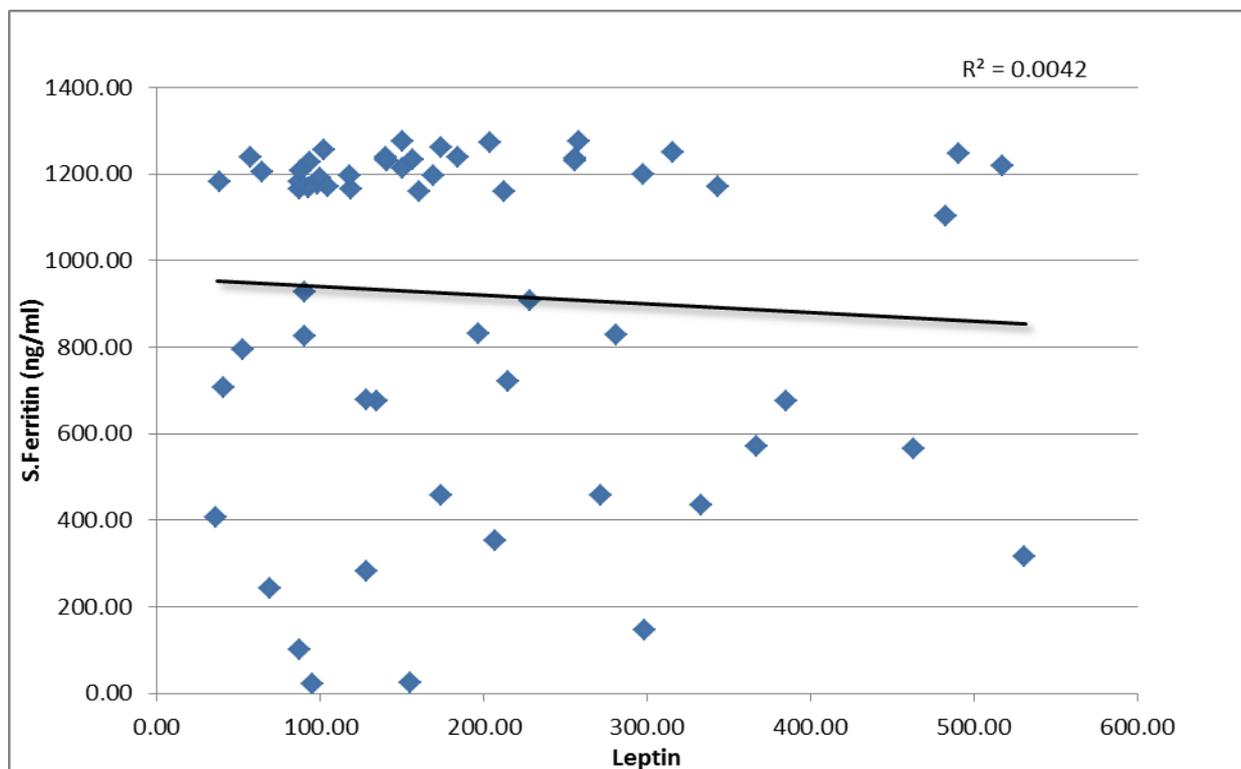
Parameters	Patients Group (Female)		Control Group (Female)		P-value
	No.	(Mean $\pm$ SD)	No.	(Mean $\pm$ SD)	
Age (years)	30	$7.39 \pm 2.22$	17	$6.58 \pm 2.25$	NS
BMI (Kg/m <sup>2</sup> )	30	$21.8 \pm 1.64$	17	$23.8 \pm 1.12$	NS
S. Leptin(pg/ml)	30	$243.40 \pm 141.14$	17	$264.53 \pm 166.04$	NS

**Table (2):** The Mean $\pm$  SD of Age, BMI and Serum Leptin of Male Patients and Control Group.

Parameters	Patients Group (Male)		Control Group (Male)		P-value
	No.	(Mean $\pm$ SD)	No.	(Mean $\pm$ SD)	
Age (years)	30	$6.74 \pm 2.51$	13	$5.46 \pm 2.70$	NS
BMI (Kg/m <sup>2</sup> )	30	$17.01 \pm 1.98$	13	$19.7 \pm 2.09$	NS
S. Leptin(pg/ml)	30	$140.32 \pm 86.03$	13	$196.31 \pm 134.75$	NS

**Table (3):** The Mean± SD of Serum Leptin, S.(cTnT) , Serum Ferritin Level, Hb and PCV of Patients and Controls Group.

Parameters	Patients Group		Control Group		P-value
	No.	(Mean± SD)	No.	(Mean± SD)	
S. Leptin (pg/ml)	60	191.86 ± 127.01	30	234.97± 154.62	NS
S. Ferritin(ng/ml)	60	922.16 ± 388.85	30	35.01 ± 23.30	< 0.001
Hemoglobin (g/dl)	60	8.27 ± 1.15	30	12.85 ± 0.53	< 0.001
PCV (%)	60	24.37± 3.67	30	38.70 ± 1.37	< 0.001



**Figure (1):** Correlation coefficient Between Serum Leptin and Serum Ferritin.

### **Discussion:**

Regarding BMI of both female and male subjects, there was a difference between the BMI of both female and male patients group less than the BMI of both female and male control group, but non-significant <sup>(10)</sup>. Many factors are contribute to stunted growth in patients

(female and male) with beta thalassemia major. The main ones were chronic anemia due to inadequate blood transfusion, endocrine disorders due to iron overload lead to deposition of iron in the body, which can damage the endocrine glands and lead to decrease

production and secretion of growth hormone (GH) and Leptin hormone, leptin can be considered one of the many metabolic signals that can regulate growth hormone (GH) secretion, leptin plays a key role in the regulation of appetite, body fat mass, and endocrine function. Leptin can predict growth retardation and delayed puberty in these patients. In addition other factor are contribute to stunted growth such as hypothyroidism, deferoxamine toxicity, inadequate treatment<sup>(11,12,13)</sup>.

In present study there was a difference between the level of leptin hormone in patients group less than control group, but non- significant. In the present study non-significant in contrast with finding<sup>(14)</sup>. In present study there was a difference between the level of leptin hormone in female patients group less than female control group but not significant. Also, there was a difference between the level of leptin hormone in male patients group less than control group, but not significant. Leptin, an adipocyte derived hormone, primarily acts in hypothalamus, toxic effect of iron overload lead to decrease leptin production, results in persistent immaturity of its hypothalamic-pituitary function<sup>(15,16)</sup>. In children with beta-thalassemia major, fat cells are not able to synthesis adequate amounts of leptin. Frequent blood transfusion and iron over load in thalassemia patients lead to chronic hemolysis, red blood cell and platelet adhesion to endothelial cells lead to reduce leptin effectiveness<sup>(17,18)</sup>.

In present study , there was a high significant difference in the level of leptin between female and male, the level of leptin was a high significant increase in female patients group as compare with male patients group

<sup>(19,20,21)</sup>. Leptin variation occur according to factors such as gender, differential distribution of adipose tissue into the visceral or subcutaneous depots, according to gender and fat distribution female have higher serum leptin concentrations than male of equivalent body weight or body mass index because leptin synthesis is greater in subcutaneous adipose tissue than in visceral adipose tissue, and the higher circulating concentration of leptin in females is due to a higher proportion of subcutaneous fat and due to depot-related differences in leptin gene expression, which is greater from subcutaneous than visceral adipocytes and due to sex hormones different in female than male which have an important role in the regulation of leptin. Also; BMI in women is more than men resulting in an increased production of leptin in women than in men<sup>(21,22,23,24)</sup>.

In present study high significant increase in serum ferritin as compare with control group. The same result high level of Serum Ferritin in patients with thalassemia was reported by other studies done before<sup>(25,26)</sup>. One mean complication of major beta thalassemia was iron overload, so that high significant increase in serum ferritin as compare with control group was observed which was related to the large amount of iron entering the body per each unit of transfused blood . In patients with major beta thalassemia, the high iron absorption results not only from blood transfusion but is due to the ineffective erythropoiesis, which is associated with an increased iron turnover<sup>(27,28)</sup>.

In present study, there was a highly significant decrease in hemoglobin concentration (Hb) and PCV in

thalassemia patients as compare to the control group<sup>(29,30)</sup>. Low Hemoglobin concentration and PCV in thalassemia patients are expected because thalassemia is a hereditary disorder characterized by abnormal production of hemoglobin, destruction of red blood cells and ineffective erythropoiesis that cause severe anemia.<sup>(31,32,33)</sup> In present study there was negative relationship between Serum Leptin level and Serum ferritin level as shown in figure (2). thus, fat cell in thalassemia patients partly is unable to produce leptin, due to the toxic effects of increased iron and ferritin levels, and the result of this increase in ferritin is reduced level of leptin<sup>(34,35)</sup>.

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