

Evaluation of Leptin Levels in Major beta-Thalassemic Patients

Choobineh H,¹ Dehghani SJ,¹ Alizadeh Sh,² Ghobadi Dana V,³ Saiepour N,⁴ Meshkani R,⁵ Einollahi N¹

¹Department of Medical Laboratory Sciences, Faculty of Para- Medicine, Tehran University of Medical Sciences

²Department of Hematology and Blood Banking, Faculty of Para- Medicine, Tehran University of Medical Sciences

³Department of Pediatrics, Faculty of Medicine, Tehran University of Medical Sciences

⁴Department of Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences

⁵Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences

Corresponding author: Hamid Choobineh

Department of Medical Laboratory Sciences, Faculty of Para- Medicine, Medical Sciences/ University Of Tehran, Poorsina Street, Ghods Street, Enghelab Avenue, Tehran, Iran

Mail code: 1417744181

Phone: +98-21-88964009

E-mail: haematology83@gmail.com

Abstract

Introduction: Leptin is an adipocyte-derived hormone. Exogenous leptin allows the recovery of the reproductive function. In humans, leptin correlates positively with the body mass index (BMI). The aim of this study was to investigate the association of leptin with the toxic effects of iron overload.

Methods: 219 Major Beta thalassemic patients (119 men, 100 women) and 137 non thalassemic individuals (86 men, 51 women) were investigated on the basis of a case control study. Data was gathered from six hospitals related to Tehran University during a seven month period (July 2006– January 2007). Blood samples of all major beta thalassemic patients who were admitted to these hospitals for recurrent blood transfusion were collected. Non thalassemic individuals were selected from outpatients who without significant medical problems, had come to these hospitals and had extra blood sample. The similarities of the two groups in age, gender and BMI also were considered.

Results: The serum leptin level median was 5.00 (interquartile range: 6.50) for major beta thalassemic patients and 6.10 (interquartile range: 7.00) for healthy individuals. Serum leptin level was significantly lower in thalassemic patients (P value <0.001). Major beta thalassemic men had significantly lower leptin level (median, interquartile range: 2.90, 3.60) than major beta thalassemic women (median, interquartile range: 6.45, 16.02; P value <0.001).

Conclusions: This study confirmed that the adipocytes of major beta thalassemic patients are unable to maintain adequate leptin production. These results suggest that adipose tissue dysfunction can be considered one of the endocrinopathies affecting major beta thalassemic patients.

Key words: Major Beta Thalassemia, Leptin, BMI

Received: 28, Aug., 2010

Accepted: 26, Sep., 2010

Introduction

Major Beta thalassemia, which is a blood disorder, results from a reduction in the synthesis of hemoglobin β chain (Hgb), and leads to an increase of the α chain in erythroid precursors. The accumulation of α chain forms an abnormal α_4 molecule which is unstable and precipitates into the plasma membrane of red blood cells (RBCs). RBCs are lysed and lost in the reticuloendothelial system; thus, major β thalassemia is classified as a hemolytic anemia.(1,2)

The severity of β thalassemia differs according to the level of β chain synthesis. Patients with major β thalassemia are dependent on recurrent blood transfusions for a long life. Blood transfusion not only causes transmission of infection diseases but also leads to premature death, as the result of an iron overload in various vital organs such as the liver and heart.(1,2) In addition to suffering from severe pain, thalassemic patients have shortened lives in comparison with healthy individuals.(1-3) Growth retardation and delay in puberty, short

stature, thinness and hypogonadism are the main problems of the thalassemic patients. The main causes of these abnormalities are not clearly understood. Hypophyseal dysfunction and decreasing gonadotropic hormones are considered as the causative factors. Relatively, the injection of gonadotropic hormones has relatively solved some of these problems.(3-6)

Leptin is a polypeptide hormone that was discovered in 1994. Leptin is a non-glycosylated protein with 146 amino acids which is positively correlated with serum lipid and the body mass index.(7-9) This hormone has diurnal variability with the peak level being at night. It appears that it is regulated by insulin.(10-11) Studies within different populations have shown that women have a higher level of leptin than men, indicating that sex hormones probably play a major role in regulating this hormone.(12-13) Leptin is the product of the obese gene (*ob*) that is mainly expressed in bone marrow (BM) and adipocytes. A decrease of leptin in knockout mice (*ob/ob*) leads to some phenotypic characteristics such as small gonads, thinness and infertility which all are reversible with hormone replacement. It also has been suggested that leptin affects hematopoietic stem cells and plays a role in erythropoiesis.(14-15)

Leptin and its receptor possess similarities to (IL)-6, IL-11, IL-12, the leukemia inhibitory factor (LIF), granulocyte-colony stimulating factor (G-CSF) and Oncostatin M (OSM).

The leptin receptor is a member of the hematopoietin receptor family, expressed in yolk sac, fetal liver, bone marrow, and hematopoietic stem cells. Leptin and erythropoietin can synergistically increase erythroid development. Knock out of leptin receptor results in impaired erythrocyte production.

Considering the abnormalities in thalassemic patients and the effects of leptin on erythropoiesis and sexual maturation, we aimed to investigate the possible relation between leptin and major beta thalassaemia in the Iranian population.

Methods

The cases of 356 individuals were researched in this case control study. This consisted of 219 thalassemic patients (119 men, 100 women) and 137 non- thalassemic individuals (86 men, 51 women) who were investigated the basis of the case control study. Data was gathered from six hospitals related to Tehran University of Medical Sciences during a seven months period (July 2006– January 2007). Age, sex, height, weight and birth date were

recorded. Blood samples of all thalassemic patients who had been admitted to these hospitals for recurrent blood transfusion were collected. Non-thalassemic individuals were selected from outpatients who without significant medical problems, had come to these hospitals and had given extra blood samples. The similarity of the two groups in age, gender and BMI was also were considered.

Blood samples (5ml) were collected without any anticoagulant agent. The serum was separated and kept at -20° until laboratory tests. Characteristics of all subjects (like age and weight) were recorded. BMI was calculated by the kg/m² formula.

Excluding criteria were a lack of extra blood samples sufficient for the tests or samples were lost while performing the tests. Written informed consent was obtained from all participants before enrollment in the study.

Leptin level in the serum was measured by Elisa kit (Diagnostic Biochem Canada Inc Cat. No: CAN-L-4260), according to kit prescription. The sensitivity of the kit was 0.5 ng/ml.

The Mann- Whitney test was used for comparisons, and the Spearman correlation coefficient was used as a test of correlation. Analyses were performed using SPSS software.

Results

A total of 356 people, consisting of 219 major beta thalassemic patients and 137 non- thalassemic individuals were subjects of the research as the basis of case control study.

Of the beta thalassemic patients, 119 (54.3%) were males and 100 (45.7%) were females. The age median was 15.3 ±7.1 in beta thalassemic patients and 15.5±4.2 in healthy individuals. Also the weight and height mean was 36.5±13.6 and 129±34.5 in beta thalassemic patients and 39.5±11.3 & 132±28.8 in healthy people respectively.

According to the filled questionnaires 2.3% of patients had been using antiviral drugs. The interval between transfusions was 3 weeks in 6% of the patients and 4 weeks in the remainder of them. All of the patients were regularly repeated transfused patients. The characteristics of the study group aged 2–29 years of age are shown in Table 1.

The Leptin level median was 5.00 ng/ml (inter-quartile range: 6.50) for major beta thalassaemia patients and 6.10 ng/ml (inter-quartile range: 7.00) for healthy individuals. Serum leptin level was significantly lower in major beta thalassaemia patients (P value <0.001). major beta thalassemic

Table 1: General characteristics of the study group

Parameter	Patients	Controls
Sex (Male/Female)	119/100	86/51
Age (mean ± SD)*	15.3 ±7.1	15.5±4.2
Weight (mean ± SD) †	36.5±13.6	39.5±11.3
Height (mean ± SD) ‡	129±34.5	132±28.8

* Year

† Kilogram

‡ Centimeter

men had significantly lower leptin level (median, inter quartile range: 2.90, 3.60) than major beta thalassaemic women (median, inter quartile range: 6.45, 16.02; P value <0.001). Leptin level and BMI had no correlation in major beta thalassaemia patients (P-value=0.67). However, leptin level and age were positively correlated among major beta thalassaemia patients (Spearman correlation coefficient= 0.234, P-values= 0.002).(Fig. 1)

Discussion

This study showed that leptin level in major beta thalassaemic patients was lower than the healthy subjects. This finding is in accordance with previous studies.(5-6, 16)

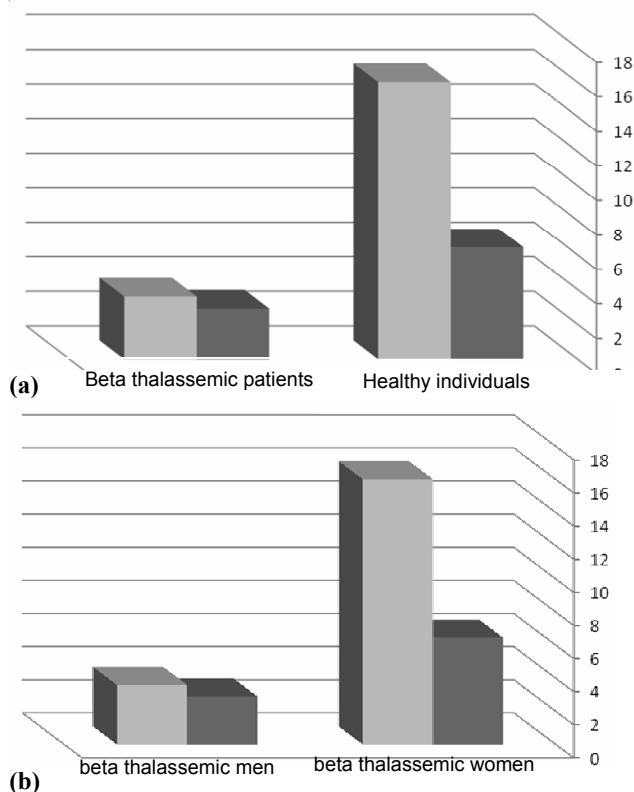


Fig 1: Leptin levels in healthy individual and Major Beta thalassaemic patients: In all bars gray bar shows median and dark bar shows inter quartile range, (a) Leptin level's median was 5.00 ng/ml (inter quartile range: 6.50) for Major Beta thalassaemic patients and 6.10 ng/ml (inter quartile range: 7.00) for healthy individuals. Serum leptin level was significantly lower in Major Beta thalassaemic patients (P value <0.001). (b) Major Beta thalassaemic men had significantly lower leptin level (median, inter quartile range: 2.90, 3.60) than Major Beta thalassaemic women (median, inter quartile range: 6.45, 16.02), (P value <0.001).

The most probable reason for such difference is the toxic effects of iron on cell membranes and proteins in the major beta thalassaemic patients, since free iron causes peroxidative damage in lipid membrane and proteins with the generation of free OH^o radicals. Thus, in an iron overload (such as in major beta thalassaemia) following the destructions of adipocyte, leptin level is decreased. Furthermore, the replacement of red BM with yellow BM which contains adipocytes (which is present in all hemolytic anemia according to the severity of the disease), can be the cause of this decrease.(2, 6, 15)

Although a positive and significant correlation between leptin and BMI has been widely observed by in other studies,(7- 9, 17, 18) we did not find any correlation between the leptin and BMI in major beta thalassaemic patients. This is in agreement with the result of some other studies.(5-6, 16) This can be attributed to a toxicity of iron overload in bone marrow and suggests a less active role of body fat mass in the regulation of circulating leptin in these patients. Animal studies have shown that iron overload and its deposition in subcutaneous layers significantly inhibits adipocyte function.(2, 15, 19)

In this study, a significant difference was found between the leptin levels of men and women in major beta thalassaemic patients, which has been confirmed by other studies.(5-6, 19) The cause of higher leptin level in major beta thalassaemic women compared to major beta thalassaemic men is considered to be related to more adipose tissue in women and also to a difference in sex hormones than is related to a diminished iron overload in women. Similar to previous studies, we also found a significant positive correlation between age and the leptin level found in major beta thalassaemic patient.(17-18) Perhaps this is related to an increase of adipose tissue with the age.

In a study by Guidance, et al, in Italy, mean values of leptin in patients and healthy subjects were 2.69 and 6.37, respectively.(5) In another study performed by Chaliouf, et al, these values were 3.21 and 8.62. In the present study, due to skewed data distribution, mean values were not used for analysis. But, it seems that the mean values of leptin level of major beta thalassaemic and non-thalassaemic individuals in our study are higher than in the previous studies. This discrepancy in leptin levels can be attributed to differences in sample size, dissimilarity of nutrition and locality and different antibody used to measure leptin. But, the most probable cause of such differences is due to the effect of leptin on hematopoiesis.(10-11) Since major beta thalassaemia is not a monotonous disease,

the severity of anemia differs according to decreasing β chain synthesis, thus leptin level differ with different levels of anemia severity. In order to clarify this theory, a patient should be selected with known severity of anemia.

In conclusion, our results from a sample of the Iranian population support the idea that major beta thalassemia is associated with lower level of leptin. In addition, confirming similar studies, this study demonstrated that women have higher levels of leptin than men. Considering this issue further, studies using synthetic hormones in animal and humans can potentially help to decrease the problems of major beta thalassemic patients.

Acknowledgements: We greatly appreciate the assistance provided by Saeed Mohammad-zadeh from department of Agricultural Extension and Education, the University of Tehran. We also thank all volunteers for their participation in the study. This research was supported by Tehran University of Medical Sciences and health Services grant (No 2740).

References

1. Loukopoulos D. Thalassemia: Genotypes and Phenotypes. *Ann Hematol*, 1991; 62:145-150.
2. Lombardo T, Tamburino C, Bartolini G, et al. Cardiac Iron Overload in Thalassaemic Patients: An Endomyocardial Biopsy Study. *Ann Hematol*, 1995; 71:135-141.
3. Low LC. Growth, Puberty and Endocrine Function in beta-Thalassaemia Major. *J Pediatr Endocrinol Metabol*, 1997; 10: 175-184.
4. De Sanctis V. Growth and Puberty and its Management in Thalassaemia. *Hormone Res*, 2002; 58 (Suppl 1):72-79.
5. Del Giudice ME, Perrotta S, Carbone MT, et al. Evaluation of Leptin Protein Levels in Patients with Cooley's Anaemia. *Bri J Haematol* 1999; 105 :839-840.
6. Karachaliou F, Vlachopapadopoulou E, Theochari M, et al. Leptin Levels in Patients with Thalassaemia Major. *Minerva Pediatr*, 2006; 58: 373-378.
7. Rosenbaum M, Nicolson M, Hirsch J, et al. Effects of Gender, Body Composition, and Menopause on Plasma Concentrations of Leptin. *J Clin Endocrinol Metabol*, 1996; 81: 3424-3477.
8. Falorni A, Bini V, Molinari D, et al. Leptin Serum Levels in Normal Weight and Obese Children and Adolescents: Relationship with Age, Sex, Pubertal Development, Body Mass Index and Insulin. *Inter J Obesity Related Metabol Disord*, 1997; 21:881-890.
9. Pelleymounter MA, Cullen MJ, Baker MB, et al. Effects of the Obese Gene Product on Body Weight Regulation in ob/ob mice. *Science* 1995; 269:540-543.
10. Gainsford T, Alexander WS. A Role for Leptin in Hemopoieses? *Mol Biotechnol*, 1999; 11:149-158.
11. Bennet BD, Solar GP, Yuan JQ, et al. A Rrole for Leptin and its Cognate Receptor in Hematopoiesis. *Curr Biol*, 1996; 6:1170-1180.
12. Saad MF, Damani S, Gingerich RL, et al. Sexual Dimorphism in Plasma Leptin Concentration. *J Clin Endocrinol Metabol*, 1997; 82:579-854.
13. Hickey MS, Israel RG, Gardiner SN, et al. Gender Differences in Serum Leptin Levels in Humans. *Biochem Mol Med*, 1996; 59:1-6.
14. El-Hazmi MA, Warsy AS, Al-Fawaz I. Iron-endocrine Pattern in Patients with beta-Thalassaemia; *J Trop Pediatr*, 1994; 40 :219-224.
15. Youson JH, Sargent PA. Iron Deposition in the Integument of Lampreys. *Anatom Record* 1984;209:461-468.
16. Dedoussis GV, Kyrtonis MC, Andrikopoulos NE, et al. Inverse Correlation of Plasma Leptin and Soluble Transferrin Receptor Levels in β -thalassaemia Patients. *Ann Hematol*, 2002; 81: 543-547.
17. Suzuki K, Ito Y, Ochiai J, et al. JACC Study Group; Relationship between Obesity and Serum Markers of Oxidative Stress and Inflammation in Japanese. *Asian Pacific J Cancer Prev*, 2003;4:259-266.
18. Matkovic V, Ilich JZ, Skugor M, et al. Leptin is Inversely Related to Age at Menarche in Human Females. *J Clin Endo Metab*, 82:3239-3245, 1997.
19. Rejholcova M, Wilhelm J, Svoboda P. Lipid Peroxidation Inhibits Norepinephrine-stimulated Lipolysis in Rat Adipocytes. Reduction of beta-adreno-ceptor number. *Biochem Biophysic Res Communications* 1988; 150:802-810.