

Evaluation of Adipokines Concentration in Iraqi Patients with Major and Minor Beta Thalassemia

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Abstract

Background: Beta thalassemia (β -thalassemia) is a type of inherited blood disorder characterized by the impaired production of beta globin chains. β -Thalassemia can be categorized into three subtypes according to symptom severity: β -thalassemia minor, β -thalassemia intermedia, and β -thalassemia major. Adipose tissue functions as an endocrine gland by synthesizing and secreting an array of bioactive peptides including leptin, adiponectin, and resistin.

Methods: We recruited 30 participants who were transfusion dependent β -thalassemia patients (major) and 30 participants who were non-transfusion dependent β -thalassemia patients (minor). The control group consisted of 20 healthy individuals. Analysis of the demographic profile, hematological profile, biochemical parameters, and serum adipokine concentrations (leptin, adiponectin and resistin) were performed for all participants.

Results: Our results showed that leptin serum levels were significantly lower in the β -thalassemia major group compared with the β -thalassemia minor group or healthy individuals, while serum levels of adiponectin were significantly higher in β -thalassemic patients compared with healthy controls. Serum levels of resistin were significantly higher in β -thalassemic patients compared with the healthy control group. A significant negative correlation was noted between adiponectin and BMI in β -thalassemic patients, whereas leptin was observed to have a significant positive correlation with BMI in the control group. Leptin was observed to have a significant negative correlation with adiponectin and ferritin in the β -thalassemia major group.

Conclusions: The changes we observed in adipokine levels may play a role in the development of the complications related to β -Thalassemia and disease severity.

Keywords: Adipokines, Adiponectin, Beta thalassemia, Leptin, Resistin

Introduction

Beta thalassemia (β -thalassemia) is an inherited hemoglobinopathy caused by the deficient or absent synthesis of beta globin chains (β -globin) required to make hemoglobin (Hb). This abnormality results in variable disease phenotypes ranging from severe chronic hemolytic anemia to clinically asymptomatic presentations (1). β -Thalassemia can be classified into three main subgroups based on symptom severity: β -thalassemia minor (β -TMI), also called β -thalassemia carrier or heterozygous β -thalassemia;

β -thalassemia intermedia; and β -thalassemia major (β -TMA), also called Mediterranean anemia or Cooley's anemia (2).

The abnormal synthesis of the β -globin present in β -thalassemia leads to excess α -globin chains that cause hemolysis and impair erythropoiesis. However, chronic vascular inflammation plays an important role in the development of complications related to this disease. It is believed that a chronic inflammatory state with elevated levels of

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Received: 29 Apr, 2020; Accepted: 10 May, 2020

inflammatory cytokines is present in patients with β -thalassemia (3).

Adipose tissue is not only a passive reservoir for energy storage but also functions as an endocrine gland by synthesizing and secreting a variety of bioactive peptides, known as adipokines (4). Adipokines participate in the etiopathogenesis of numerous metabolic, vascular, and inflammatory disorders (5). Adipokines include adiponectin, resistin, leptin, visfatin, plasminogen activator inhibitor type 1 (PAI-1), tumour necrosis factor alpha (TNF- α), interleukin (IL)-6, and IL-8 (6).

Leptin is a 16 KD polypeptide hormone with a mature sequence consisting of 146 amino acids (7). Leptin is a multifunctional hormone playing a role in many different biological functions including the regulation of energy homeostasis, angiogenesis, and inflammation (8). Recent research suggests that leptin may also affect hematopoiesis and play a role in erythropoiesis (9).

Adiponectin (ADP) is a 3KD long polypeptide with a mature sequence consisting of 244 amino acids. Adiponectin modulates several metabolic processes, including glucose regulation and lipid metabolism. However, adiponectin is also involved in attenuating the inflammatory response, oxidative stress, and cytokine production (10).

Resistin is a 12.5 KD cysteine rich peptide with a mature sequence consisting of 108 amino acids. Resistin belongs to the hormone family of resistin-like molecules (RELM) that are involved in the regulation of the inflammatory process (8). Recent studies have indicated a causal relationship between resistin and systemic inflammation, particularly in the vascular endothelium (11).

The aim of this study was to evaluate the levels of the adipokines leptin, adiponectin, and resistin, in Iraqi patients with major and minor β -thalassemia and determine potential correlations with disease severity.

Materials and Methods

Study participants

The present study included 60 patients diagnosed with β -thalassemia divided into two groups: 30 patients with transfusion dependent thalassemia

(15 males and 15 females) ranging from 8-30 years of age, and 30 patients with non-transfusion dependent thalassemia (15 males and 15 females) ranging from 9-31 years of age. This study also included 20 healthy individuals (10 males and 10 females) as the control group with ages ranging from 8-31 years. The participants were recruited from Abin Al-Baladi hospital, Hereditary Blood Disorder Centre, Baghdad city. This study was approved by the Department of Chemistry, College of Science, Al-Nahrain University, Baghdad, and by the Research Ethics Committee of the Iraqi ministry of health, Iraq. Patients with transfusion dependent thalassemia were on iron chelator therapy (Deferasirox, Exjade, 10-30 mg/Kg/day).

Exclusion criteria

Participants with the following criteria were excluded from the current study, as these factors may have an impact on adipokine concentrations: smoking, history of hypertension, heart failure, diabetes mellitus, hypothyroidism, and hepatic or renal diseases, patients taking any medication other than iron chelators or splenectomized, and drug use or obesity in any of the healthy individuals.

Sample collection

Eight milliliters of venous blood was drawn from patients and healthy individuals after an overnight fasting of 8-12 h. Blood samples from patients with β -thalassemia major were obtained prior to receiving their blood transfusion. The blood samples were divided into two tubes: two milliliters into ethylene diamine tetra acetic acid (EDTA) tubes for hematological measurement and six milliliters into gel tubes that were left for 15 minutes to clot. Serum was separated from the blood samples via 15-minute centrifugation at 1814 x g in room temperature. The extracted serum was divided into aliquots and stored at -70 °C until assayed.

Measurement of Body Mass Index (BMI)

BMI was measured by dividing weight (in Kilograms, Kg) by height squared (in meter, m) for each participant.

Measurement of hematological profile

Complete blood count (CBC) was measured using flow cytometry in hematology (12). Automated Hematology Analyzer system (Sysmex KX-21N, Sysmex Corporation, Kobe, Japan) was used to measure red blood cell (RBC) count, hemoglobin (Hb), and hematocrit (Hct).

Biochemical analysis

Serum ferritin concentrations were measured using the ElectroChemiLuminescence method with Roche Cobas E411 autoanalyser system (Roche-Hitachi Diagnostics, Japan). Serum iron concentrations were measured with the Ferene kit supplied from BIOLABO Company (French manufacturer of Reagents for Medical Biology, France) with MINDRAY semi-auto chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics Co. LTD., China).

Serum adipokine concentrations were assayed using enzyme linked immune sorbent assay (ELISA) kits according to the manufacturer's recommended procedure. Human ADP ELISA Kit was provided by (MyBioSource, USA), while human leptin and resistin ELISA kits were provided by (Komabiotech, South Korea).

Statistical analysis

Statistical analysis of the demographic and biochemical data was performed using the GraphPad Prism software version 7.04 (San Diego, California, USA). One-way ANOVA was performed to assess mean \pm standard deviation and statistical significance (p-value) between the means of the three studied groups. Correlations between variables were estimated using Spearman's correlation coefficient. P value < 0.05 was considered statistically significant.

The difference letters (a, b, and c) that present in the same row in the tables mean a significant difference in the parameter between groups.

Results

Table (1) shows the demographic data of the three studied groups (β -TMA, β -TMi, and control). The results obtained from the preliminary analysis shown in table (1) indicated that there were no significant differences between both β -thalassemia groups and the control group regarding age and gender ($p > 0.05$), while there was a significant difference ($p < 0.0001$) in BMI between the β -TMA group and the control or β -TMi group.

Table 1. Statistical analysis of demographic characteristics among the β -TMi, β -TMA, and control groups.

| Variable | Control | β -TMi | β -TMA | P-value | Significant |
|--------------------------|-----------------------------|-----------------------------|-----------------------------|------------|-------------|
| Age (year) | 18.6 \pm 7.4 | 19.1 \pm 6.5 | 18.1 \pm 6.5 | 0.8641 | NS |
| Gender (Male/Female) | 10/10 | 15/15 | 15/15 | - | - |
| BMI (Kg/m ²) | 22.9 \pm 5.3 ^a | 23.8 \pm 5.4 ^a | 16.9 \pm 3.3 ^b | < 0.0001 | HS |

All values are shown as mean \pm SD (Standard Deviation). **S:** p-value < 0.05 (Significant), **NS:** p-value > 0.05 (Non-Significant), **HS:** p-value < 0.0001 (Highly Significant).

Table (2) shows laboratory data from the blood analysis among the three groups. Our findings indicate that there were significant differences ($p < 0.0001$) regarding the RBC count, Hb, and Hct among all groups. Additionally, there were significant differences ($p < 0.0001$) between the β -TMA group and the β -TMi or control groups regarding serum levels of ferritin and iron.

Table (3) shows the different adipokine concentrations among the three groups. Statistical analysis revealed that serum levels of leptin were significantly lower ($p < 0.0001$) in the β -TMA group compared with the other two groups.

Moreover, there was no significant difference ($p = 0.3265$) between the β -TMi and control groups with respect to leptin serum levels. In contrast to leptin, serum levels of adiponectin were significantly different ($p < 0.0001$) among all three groups. Furthermore, serum levels of resistin were significantly higher ($p < 0.0014$) in both the β -TMA and β -TMi groups compared to controls.

We determined the Spearman's correlation coefficient among the different variables in the study. For leptin and ferritin, we found a significant negative correlation ($r = -0.491$, $p = 0.041$) in the β -TMA group. A significant positive

correlation was found between leptin and BMI ($r=0.481$, $p=0.034$) in the control group. A significant negative correlation was found between ADP and BMI in the β -TMa group ($r=-0.541$, $p=$

0.021) and the β -TMi group ($r=-0.411$, $p=0.041$). Additionally, a significant negative correlation was found between ADP and leptin ($r=-0.546$, $p=0.002$) in the β -TMa group.

Table 2. Comparison of hematological profiles between the β -TMi, β -TMa, and control groups.

| Parameter | Control | β -TMi | β -TMa | p-value | Sign |
|-----------------------------------|--------------------------------|--------------------------------|-----------------------------------|---------|------|
| RBC ($\times 10^6/\mu\text{L}$) | 4.7 \pm 0.5 ^a | 5.5 \pm 0.8 ^b | 2.8 \pm 0.4 ^c | <0.0001 | HS |
| Hb (g/dL) | 13.6 \pm 1.3 ^a | 11.1 \pm 1.6 ^b | 7.7 \pm 0.9 ^c | <0.0001 | HS |
| Hct (%) | 40.8 \pm 4.3 ^a | 35.3 \pm 5.3 ^b | 22.4 \pm 2.8 ^c | <0.0001 | HS |
| Ferritin (ng/mL) | 50.57 \pm 58.03 ^a | 78.99 \pm 95.14 ^a | 3859.40 \pm 2550.7 ^b | <0.0001 | HS |
| Iron ($\mu\text{g/dL}$) | 114.6 \pm 47.7 ^a | 128.1 \pm 42.4 ^a | 210.2 \pm 65.9 ^b | <0.0001 | HS |

a,b,c: The different letters in the same column indicate a significant difference of these parameters between the groups. **RBC**: red blood cell; **Hb**: Hemoglobin; **Hct**: Hematocrit.

Table 3. Comparison of adipokine concentrations among the β -TMi, β -TMa, and control groups.

| Parameter | Control | β -TMi | β -TMa | p-value | Sign |
|------------------|---------------------------------|---------------------------------|--------------------------------|---------|------|
| Leptin (pg/mL) | 1409.0 \pm 443.3 ^a | 1114.4 \pm 628.7 ^a | 317.1 \pm 287.5 ^b | <0.0001 | HS |
| ADP (ng/mL) | 35.4 \pm 14.6 ^a | 57.9 \pm 16.5 ^b | 74.5 \pm 11.6 ^c | <0.0001 | HS |
| Resistin (pg/mL) | 1730.4 \pm 41.2 ^a | 1767.2 \pm 42.3 ^b | 1773.7 \pm 40.2 ^b | <0.0014 | S |

ADP: adiponectin.

Discussion

The objective of this study was to investigate the serum levels of leptin, adiponectin, and resistin in patients diagnosed with major and minor β -thalassemia to explore the role of adipokines in the pathophysiology of this disease.

We found serum levels of leptin to be significantly lower ($p<0.0001$) in the β -TMa group compared to the other two groups, findings which corroborate previous research (13-14). Serum levels of adiponectin were found to be significantly higher ($p<0.0001$) in the patients with β -thalassemia (with significant differences between both patient groups) compared to the healthy individuals, which are similar to findings from a previous study (15). Moreover, serum levels of resistin were significantly higher ($p=0.0014$) in β -thalassemic patients (with no significant difference between each patient group, $p=0.8653$) than in the healthy control group, which is in line with observations from previous work (15-16). The endocrine function of adipose tissue involves the synthesis and secretion of adipokines. These signaling proteins participate in the etiopathogenesis of numerous metabolic, vascular,

and inflammatory disorders (17). The findings in the current study further support the mounting evidence for a role of adipose tissue-associated inflammation in the pathogenesis of β -thalassemia complications (3). The anomalies in the individual's beta globin levels alone are unable to fully explain the diversity of complications experienced by patients with β -thalassemic, such as cardiovascular issues or chronic vascular inflammation. Therefore, increased adhesiveness of erythrocytes and platelets to endothelial cells due to chronic hemolysis, chronic iron overload, and oxidative stress are considered potential factors that contribute to the endothelial damage and vascular inflammation seen in these patients.

Our study found patients in the β -TMa group to have low serum levels of leptin and a negative correlation between serum leptin and ferritin levels, which corroborates findings from a previous study (14). These observations may be explained by the effects of iron overload in patients with β -thalassemic. The toxic effect of iron overload involves the destruction of adipocytes by the release of free iron which causes peroxidative

damage to the adipocyte lipid membrane and proteins due to the generation of O₂ free radicals (18). Previous animal studies examining iron overload have shown that the deposition of iron in adipocytes within the subcutaneous layer can significantly inhibit adipocyte function (19-20). These findings support an association between iron overload and adipose tissue dysfunction that could be considered one of the endocrinopathies present in β -thalassemia. A positive correlation between leptin and BMI was found only in our healthy control group, which is in agreement with findings from previous research (21). This association may be explained by the fact that the increased fat mass leads to an increase in the production of leptin (22). These findings may help explain the underlying cause contributing to the decreased serum levels of leptin observed in β -thalassemic patients.

In the current study, serum levels of ADP were found to be significantly higher in β -thalassemic patients than in the control group. No significant correlation between serum ADP and ferritin levels were found in any of the three groups, which is in line with previous research (23). Contrary to what was expected, we found a significant negative correlation between ADP and BMI in patients with β -thalassemia major ($r = -0.541$, $p = 0.021$) and β -thalassemia minor ($r = -0.411$, $p = 0.041$). Moreover, there was a significant negative correlation between ADP and leptin ($r = -0.546$, $p = 0.002$) in the group of patients with β -thalassemia major. These observations regarding the correlation between ADP with BMI and leptin may be a result of adipose tissue being unable to provide sufficient leptin production due to the toxic effects of iron overload on adipocytes in patients with β -thalassemia. However, ADP may be secreted by cells other than adipocytes. Recent studies suggest that ADP is produced by synovial fibroblasts (24), osteoblasts (25), and skeletal muscle cells and even endothelial cells (26). Vascular endothelial cells can contribute to atherogenesis and are mediators in the inflammatory response due to their ability to produce and detect cytokines, including endothelium-1 (ET-1) which has vasoconstrictive and pro-inflammatory effects, and their expression of adhesion molecules (27). A moderate positive

correlation that was found between ADP and ET-1 in a previous study (23). Moreover, ET-1 participates in the secretion of ADP in a time and dosage-dependent manner (28). However, activated or damage endothelial cells as a result of chronic hemolysis, chronic iron overload or oxidative stress in β -thalassemic patients could be directly related to the increased ADP or indirectly through the regulation of ET-1 (23). Therefore, ADP levels in β -thalassemic patients could indicate the patient's level of endothelial damage and cardiovascular risk.

Our study also found serum levels of resistin to be significantly higher in β -thalassemic patients compared with the healthy control group. Resistin is similar to ADP in that it is considered a pro-inflammatory adipokine and appears to have immunomodulatory potential (29). A previous study indicated that recombinant human resistin enhances the secretion of pro-inflammatory adipokines such as TNF-alpha, IL-6, and IL-12 in both murine and human macrophages (30). In a separate study, human recombinant resistin was observed to activate human endothelial cells and cause an increase in the expression of ET-1, several different adhesion molecules, and chemokines (29). Based on predicting the severity of coronary atherosclerosis (31), the increase in inflammatory mediator levels cause an increase in the level of resistin. In our study, resistin was found to be significantly higher in β -thalassemic patients (with no significant differences between major and minor β -thalassemia groups) compared to the control group. This finding further supports the hypothesis that changes in ADP and resistin concentrations occur according to disease severity.

In conclusion, our findings show leptin levels to be significantly decreased in β -thalassemic patients, while ADP and resistin levels were significantly higher compared to controls. These changes in adipokine levels may have an important role in the development of inflammation-induced complications associated with β -thalassemia.

Acknowledgment

The authors would like to acknowledge staff in Abin Al-Baladi hospital for their assistance in with this work.

References

1. Angastiniotis M, Lobitz S. Thalassemias: An overview. *Int J Neonatal Screen*. 2019;5(1):1-11.
2. Wafaa FA-M. The Beta-Thalassemia. *Sci J Med Res*. 2017;1(1):24-30.
3. Kanavaki I, Makrythanasis P, Lazaropoulou C, Tsironi M, Kattamis A, Rombos I, et al. Soluble endothelial adhesion molecules and inflammation markers in patients with β -thalassemia intermedia. *Blood Cells, Mol Dis*. 2009;43(3):230-234.
4. Jakovljević B, Paunović K, Stojanov V. Adipose tissue as an endocrine organ. *Srp Arh Celok Lek*. 2005;133(9-10):441-5.
5. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol*. 2011;11(2):85–97.
6. Shahramian I, Akhlaghi E, Ramezani A, Rezaee A, Noori N, Sharafi E. Adipokines and the endocrine role of adipose tissues. *Metabolic Control*. 2015;265-282.
7. Ahima RS, Flier JS. Leptin. *Annu Rev Physiol*. 2000;62:413-437.
8. Banks WA. The many lives of leptin. *Peptides*. 2004;25(3):331-338.
9. Fantuzzi G, Faggioni R. Leptin in the regulation of immunity, inflammation, and hematopoiesis. *J Leukoc Biol*. 2000;68(4):437-46.
10. Lee S, Kwak H-B. Role of adiponectin in metabolic and cardiovascular disease. *J Exerc Rehabil*. 2014;10(2):54-59.
11. Abate N, S Sallam H, Rizzo M, Nikolic D, Obradovic M, Bjelogrić P, et al. Resistin: an inflammatory cytokine. Role in cardiovascular diseases, diabetes and the metabolic syndrome. *Curr Pharm Des*. 2014;20(31):4961-9.
12. Brown M, Wittwer C. Flow cytometry: Principles and clinical applications in hematology. *Clin Chem*. 2000;46(8):1221-1229.
13. Choobineh H, Dehghani SJ, Alizadeh S, Ghobadi Dana V, Saiepour N, Meshkani R, et al. Evaluation of leptin levels in major beta-thalassemic patients. *Iran J Ped Hematol Oncol*. 2009;3(4):1-4.
14. Adel AER, Maaly M Mabrouk, MD. *IMBMD*. Study of Serum Leptin in Children with Beta Thalassemia: Correlation with Iron Overload. *Med J Cairo Univ*. 2018;86(9):3037-3045.
15. Enli Y, Balci YI, Gönen C, Uzun E, Polat A. Adipocytokine concentrations in children with different types of beta-thalassemia. *Scand J Clin Lab Invest*. 2014;74(4):306-11.
16. Deeb MM, Dawoud AA, El-Hawry MA, Wasel YFM, El Sayed AE. Study of adipocytokines (visfatin and resistin) levels in children with β -thalassemia major and intermedia. *Menoufia Med J*. 2019;32(3):1051-1058.
17. Piya MK, McTernan PG, Kumar S. Adipokine inflammation and insulin resistance: The role of glucose, lipids and endotoxin. *J Endocrinol*. 2013;216(1).
18. Dayer D, Salahcheh M, Jazayeri SMH, Kaydani GA, Kadkhodaei Elyaderani MK, Shaneh S. Thyroid stimulating hormone and leptin levels and severe growth retardation among Beta- thalassemic patients. *Pak J Med Sci Q*. 2012;28(3):421-423.
19. Youson JH, Sargent PA. Iron deposition in the integument of lampreys. *Anat Rec*. 1984;209(4):461-8.
20. Rejholcova M, Wilhelm J, Svoboda P. Lipid peroxidation inhibits norepinephrine-stimulated lipolysis in rat adipocytes. Reduction of beta-adrenoceptor number. *Biochem Biophys Res Commun*. 1988;150(2):802-810.
21. Elsayh KI, Mohammed WS, Zahran AM, Saad K. Leukocytes apoptosis and adipocytokines in children with beta thalassemia major. *Clin Exp Med*. 2016;16(3):345-50.
22. Suzuki K, Ito Y, Ochiai J, Kusuhara Y, Hashimoto S, Tokudome S, et al. Relationship between obesity and serum markers of oxidative stress and inflammation in Japanese. *Asian Pacific J Cancer Prev*. 2003;4(3):259-66.
23. Chaliasos N, Challa A, Hatzimichael E, Koutsouka F, Bourantas DK, Vlahos AP, et al. Serum adipocytokine and vascular inflammation marker levels in beta-thalassaemia major patients. *Acta Haematol*. 2010;124(4):191-6.
24. Ehling A, Schäffler A, Herfarth H, Tamer IH, Anders S, Distler O, et al. The potential of adiponectin in driving arthritis. *J Immunol*. 2006;176(7):4468-78.

25. Berner HS, Lyngstadaas SP, Spahr A, Monjo M, Thommesen L, Drevon CA, et al. Adiponectin and its receptors are expressed in bone-forming cells. *Bone*. 2004;35(4):842-9.
26. Delaigle AM, Jonas J-C, Bauche IB, Cornu O, Brichard SM. Induction of adiponectin in skeletal muscle by inflammatory cytokines: *in vivo* and *in vitro* studies. *Endocrinology*. 2004;145(12):5589-97.
27. Hartge MM, Unger T, Kintscher U. The endothelium and vascular inflammation in diabetes. *Diabetes Vasc Dis Res*. 2007;4(2):84-8.
28. Juan C-C, Chuang T-Y, Chang C-L, Huang S-W, Ho L-T. Endothelin-1 regulates adiponectin gene expression and secretion in 3T3-L1 adipocytes via distinct signaling pathways. *Endocrinology*. 2007;148(4):1835-42.
29. Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ, Lazar MA. An inflammatory cascade leading to hyperresistinemia in humans. *PLoS Med*. 2004;1(2):e45.
30. Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ. Human resistin stimulates the pro-inflammatory cytokines TNF- α and IL-12 in macrophages by NF- κ B-dependent pathway. *Biochem Biophys Res Commun*. 2005;334(4):1092-101.
31. Szapary PO, Bloedon LT, Samaha FF, Duffy D, Wolfe ML, Soffer D, et al. Effects of pioglitazone on lipoproteins, inflammatory markers, and adipokines in nondiabetic patients with metabolic syndrome. *Arterioscler Thromb Vasc Biol*. 2006;26(1):182-8.