Al-Nahrain Journal of Science
ANJS, Vol. 28(1), March, 2025, pp. 1–7

Evaluation of Serum Leptin and Ferritin Levels in Females with Beta Thalassemia Major

Fatima A. Abdulhaleem[1], Alaa Hussein J. Al-Qaisi[1], Dalia M. Jamil[1], Amamer M. Redwan[2], Nur Wahida Zulkifli[3]

[1] Department of Chemistry, College of Science, Al-Nahrain University, Baghdad, Iraq.
[2] Department of Chemistry, Faculty of Science, Bani Waleed University, Bani Waleed, Libya.
[3] Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, FF1, Level 11, Bandar Puncak Alam 42300 Puncak Alam, Selangor, Malaysia.

Article's Information

Received: 24.04.2024
Accepted: 07.07.2024
Published: 15.03.2025

Keywords:
Adipokines
Leptin
Iron overload
Splenectomized
Beta thalassemia major

Abstract

Beta thalassemia is an inherited blood disorder caused by inadequate or absence synthesis of ß-globin chains. It is classified into three subgroups: ß-thalassemia minor, ß-thalassemia intermedia, and ß-thalassemia major. Beta thalassemia patients suffer from chronic hemolytic anemia and consequently, require frequent blood transfusions, which however can cause iron overload in tissues and organs. The accumulation of iron can cause severe complications such as endocrine dysfunction and chronic liver diseases. Leptin, a polypeptide hormone secreted by adipose tissue and plays an important role in maintaining body weight. This study aimed to evaluate serum leptin and ferritin levels in females with beta thalassemia major. The study included 35 splenectomized thalassemia patients and 35 healthy controls. Anthropometric profile, hematological profile, and serum ferritin and leptin levels were determined for all participants. The results showed a significant decrease in body mass index (BMI), red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), and leptin in thalassemia patients compared to the healthy controls. In contrast, there was a significant increase in serum ferritin in thalassemia patients compared to the healthy controls. It is concluded that patients with beta thalassemia major had considerably lower serum leptin levels. The increased blood ferritin levels in these patients suggest that the malfunction of adipose tissue brought on by iron overload is probably linked to this drop in leptin.

http://doi.org/10.22401/ANJS.28.1.01
*Corresponding author: fatimaazzam07@gmail.com

1. Introduction

Beta thalassemia is an inherited blood disorder characterized by inadequate or absence beta globin chains synthesis, essential for hemoglobin production. The pathological condition gives rise to diverse disease phenotypes ranging from clinically asymptomatic presentations to severe chronic hemolytic anemia [1]. Based on the severity of symptoms, beta thalassemia is classified into three subgroups: ß-thalassemia minor, also referred to as "heterozygous ß-thalassemia" or "ß-thalassemia carrier", ß-thalassemia intermedia, and ß-thalassemia major, commonly known as "Mediterranean anemia" or "Cooley's anemia" [2]. In beta thalassemia, aberrant ß-globin synthesis results in excess α-globin chains, leading to hemolysis and impaired erythropoiesis [3]. This impairment leads to premature erythrocyte destruction, chronic anemia, extra medullary hematopoiesis and bone marrow enlargement in severe cases [4]. Patients with ß-thalassemia suffer from severe chronic hemolytic anemia and therefore,

require frequent blood transfusions starting in early childhood. Iron chelation therapy (ICT) is commonly used alongside chronic blood transfusions to prevent iron overload complications [5]. While blood transfusions are a crucial therapeutic approach for individuals with beta thalassemia, studies have demonstrated that repeated transfusions may cause an excess of iron in tissues and organs, disrupting their normal functions. The accumulation of iron in various tissues can lead to complications such as endocrine dysfunction, arrhythmia, heart failure, infections, and chronic liver diseases [6]. The clinical manifestations of beta thalassemia patients include liver and gall bladder alterations, spleen abnormalities, skull and bone deformities, and pale skin due to anemia and jaundice. Iron overload is the primary complication of ß-thalassemia patients and requires careful management [7]. Adipose tissue serves as an endocrine gland by producing and secreting a group of biologically active peptides called adipokines, in addition to acting as a passive energy storage reservoir [8]. Adipokines play important roles in regulating inflammation, immunity, metabolism, cardiovascular health, and many other physiological functions [9]. Adipokines include leptin, adiponectin, resistin, visfatin, interleukin (IL-6), interleukin (IL-8), and many others [10]. Leptin is one of the most important hormones derived from the adipose tissue. Leptin was first identified almost at the end of 1994 and its name was derived from the ancient Greek word leptos which means " thin ". The term " Ob protein " refers to leptin (Ob for obese). Leptin has a direct correlation with the quantity of body fat and body mass index (BMI) [11].

Leptin is a 16 KD polypeptide hormone with a mature sequence comprising 146 amino acids and in human the Ob/Lep gene is found on chromosome 7. Leptin's structure places it in the same family of long chain helical cytokines such as IL-6, IL-11, IL-12, and leukemia inhibitory factor (LIF) [12, 13]. Leptin is produced by white adipose tissue (WAT) and primarily by adipocytes. However, leptin can be produced in small amounts and under specific circumstances by other cells rather than adipocytes such as the placenta, mammary epithelium, gastric mucosa, bone marrow, skeletal muscle, hypothalamus, and pituitary [14]. Leptin is involved in several processes including the metabolism of glucose, the synthesis of glucocorticoids, the release of cytokines, the control of the hypothalamic pituitary adrenal system, angiogenesis, and reproduction [15]. However, the structural homology of leptin's receptors with those of class I cytokine receptors, which regulate hematopoiesis, suggests that leptin may have an impact on the production of red blood cells. According to previous research, leptin and its receptors are found in bone marrow and have an impact on hematopoietic stem cells (HSCs), which are necessary for the production of red blood cells (RBCs) [16].

This study aimed to evaluate serum leptin and ferritin levels in females with beta thalassemia major.

2. Materials and Methods

The present study involved 70 participants (aged 18-30) years divided into two groups: 35 patients with splenectomized beta thalassemia major (group I), and 35 healthy controls (group II). The patients were recruited from Al-Karama Teaching Hospital, Hereditary Blood Disorder Center, Baghdad. This work was approved by Al-Nahrain University, College of Science, Department of Chemistry, Baghdad, and by the Iraqi Ministry of Health's Research Ethics Committee, Iraq (Ethical No.:2023194).

2.1. Exclusion Criteria

Individuals with diabetes mellitus, hypertension, heart failure, hepatitis, or hereditary blood disorders other than beta thalassemia were excluded from this study.

2.2. Blood Sample Collection

A total of 8 ml of venous blood was collected from both patients and healthy controls following 8-12 hour of an overnight fasting. For patients with beta thalassemia major, blood samples were collected prior to blood transfusion. The blood samples were divided into two tubes: (2 ml) were placed in EDTA tubes for hematological analysis, and (6 ml) were placed in gel tubes and allowed to coagulate for 15 minutes. The samples were then centrifuged at room temperature for 15 minutes at 5500 rpm to separate the serum. The serum was divided into aliquots and stored at -20 °C until analysis.

2.3. Determination of Body Mass Index (BMI)

BMI was calculated for all participants by dividing their weight in kilograms (kg) by their height squared in meters (m).

2.4. Determination of Hematological Profile Count

Red blood cell (RBC) count, hemoglobin (Hb) level, hematocrit (Hct) percentage, and mean corpuscular volume (MCV) level were measured using the Swelab Alpha (Swedish) automated hematology analyzer via flow cytometry [17].

2.5. Determination of Biochemical Tests

Serum ferritin levels were measured using the electrochemiluminescence technique with the Roche Cobas E411 (Germany) autoanalyzer system. Serum leptin levels were measured using the enzyme linked immune sorbent assay (ELISA) technique (MyBioSource, USA).

2.6. Statistical Analysis

Statistical analysis was performed using Graph Pad Prism version 8.3. Both analytical and descriptive statistics were calculated including the student t-test, and the mean ± standard deviation (SD). A P-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

The anthropometric, hematological, and biochemical parameters in splenectomized ß- thalassemia major and the healthy controls are compared and presented in table 1. The study found no significant difference in age between splenectomized ß-thalassemia major and the healthy controls. However, there was a significant decrease in BMI, RBC, Hb, Hct, MCV, and leptin levels in splenectomized ß-thalassemia major compared to the healthy controls. In contrast, there was a significant increase in serum ferritin level in splenectomized ß-thalassemia major compared to the healthy controls.

Table 1. Comparison of several parameters between splenectomized ß-TMa and the healthy controls.

Parameters	Splenectomized ß-TMa	Healthy Controls	P-value
Age (years)	24.43 ± 3.97	24.30 ± 2.507	0.3838
BMI (kg/m^2)	20.24 ± 0.9887[a]	24.60 ± 2.302[b]	<0.0001
RBC (*10^6/µL)	3.255 ± 0.3618[a]	4.454 ± 0.2944[b]	<0.0001
Hb (g/dL)	8.840 ± 0.5405[a]	12.46 ± 1.038[b]	<0.0001
Hct (%)	26.24 ± 2.237[a]	38.75 ± 2.943[b]	<0.0001
MCV (fL)	75.44 ± 2.882[a]	88.31 ± 2.837[b]	<0.0001
Ferritin (ng/ml)	2394 ± 1668[a]	58.84 ± 28.52[b]	<0.0001
Leptin (ng/ml)	2.015 ± 0.7518[a]	3.534 ± 0.6322[b]	<0.0001

The small letters (a, and b) in the table that appear in the same row indicate that there is a significant difference in the parameter between the two groups.

BMI: Body Mass Index, RBC: Red Blood Cell, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean Corpuscular Volume.

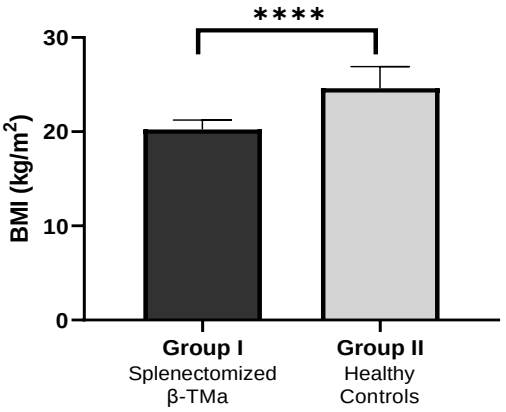

Figure 1. BMI level in the studied groups.

Statistical significant considered as:
Non-Significant when P-value >0.05.
Significant when P-value <0.05.
Highly Significant when P-value <0.0001.

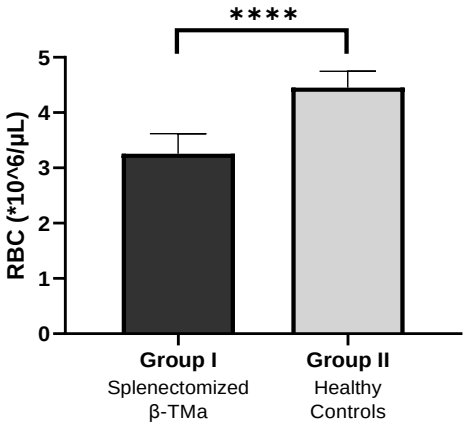

Figure 2. RBC level in the studied groups.

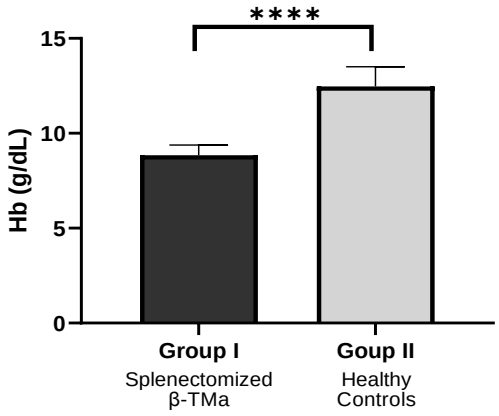

Figure 3. Hb level in the studied groups.

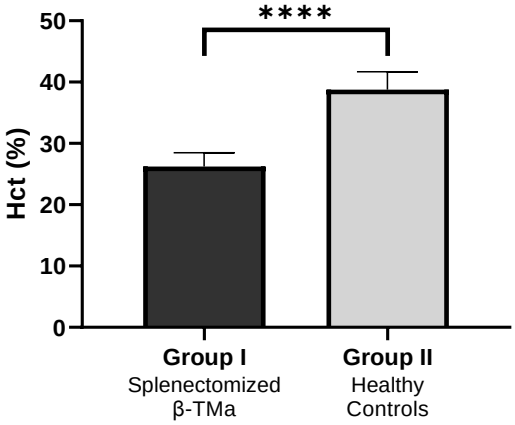

Figure 4. Hct level in the studied groups.

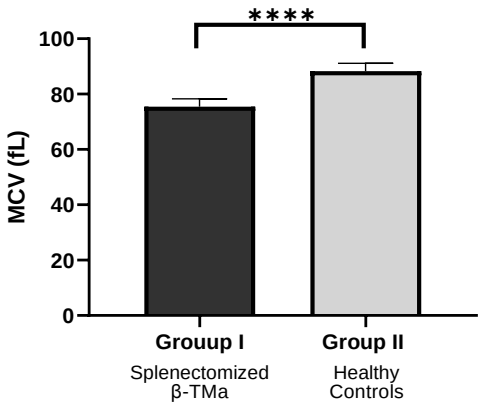

Figure 5. MCV level in the studied groups.

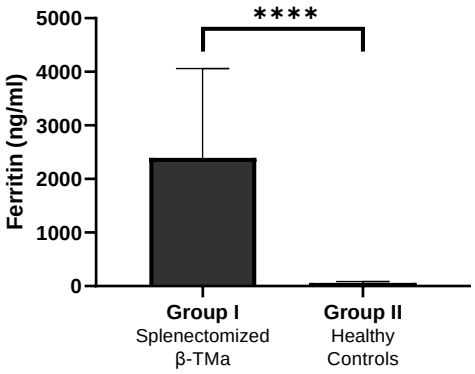

Figure 6. Serum ferritin level in the studied groups.

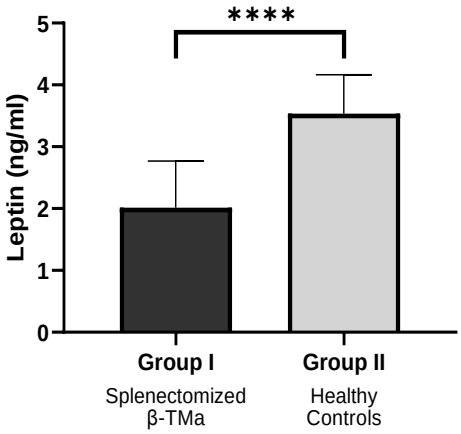

Figure 7. Leptin level in the studied groups.

The current study aimed to evaluate serum leptin and ferritin levels in females with beta thalassemia major. The findings showed a significantly lower level of BMI in splenectomized beta thalassemia major compared to the healthy controls. This decrease in BMI level can be attributed to factors such as poor nutritional support, chronic disease, endocrine abnormalities, and low hemoglobin level [18]. This finding aligns with the results of (Al-Naama et al., 2016) who reported a significantly lower level of BMI in splenectomized beta thalassemia major patients compared to the healthy controls [19]. Conversely, this result contradicts with the findings of (El-Deen et al., 2014) who observed no significant difference in BMI level between splenectomized beta thalassemia major patients and the healthy controls [20].

Thalassemia is a hereditary blood disorder that disrupts the erythrocyte production process [21]. The findings revealed a significantly lower level of red blood cell (RBC), which can be attributed to the defective hemoglobin chains that cause abnormalities in RBC generation by hematopoietic stem cells in the bone marrow [22]. This results in agreement with what is found by (Khawaji et al., 2020) who demonstrated that there was a significantly lower level of RBC in beta thalassemia major patients compared to the healthy controls [23]. Also, the findings showed that there was a significantly lower level of hemoglobin (Hb), which can be attributed to microcytic anemia that is characterized by irregularly shaped and reduced RBC, which leads to hypoxia in the blood and eventually in the tissues, in which hemoglobin (Hb) level will be decreased. This results in agreement with what found by (Tari et al., 2018), and (Arab-Zozani et al., 2021) [24, 25]. The findings revealed that there was a significantly lower level of Hct due to a mutation in the gene that produces the protein globin, which causes RBC to become fragile and rupture before they mature [26]. This results in agreement with what found by (Roth et al., 2018) who demonstrated that there was a significantly lower level of Hct in beta thalassemia major patients compared to the healthy controls [27]. Also, the findings showed that there was a significantly lower level of MCV because patients with beta thalassemia major suffer from chronic anemia caused by low oxygen level in their blood. These results in agreement with what is found by (Alathari and Mahdi et al., 2019) who demonstrated that there was a significantly lower level of MCV in beta thalassemia major patients compared to the healthy controls [28]. The outcomes showed that there was a significantly higher level of serum ferritin, which can be attributed to recurrent blood transfusions, increased iron absorption from the gastrointestinal tract, and frequent destruction of red blood cells in the spleen [29]. This results in agreement with what is found by (Hagag et al., 2018) who demonstrated that there was a significantly higher level of serum ferritin in beta thalassemia major patients compared to the healthy controls [30].

The outcomes revealed that there was a significantly lower level of leptin in beta thalassemia major patients compared to the healthy controls, which can be attributed to the fact that iron overload and iron accumulation in fat cells can cause harmful consequences of iron such as the production of free radicals that suppress the action of adiposities. In addition, the damage of the membrane of fat cell and malfunction of adipose tissue causes a significantly lower level of leptin in these patients [31]. This results in agreement with what found by (Harbi et al., 2020) who demonstrated that there was a significantly lower level of leptin in beta thalassemia major patients compared to the healthy controls [32]. Also, the study conducted by (Al-Fatlawi et al., 2022) demonstrated that beta thalassemia major patients have a significantly lower level of leptin compared to the healthy controls [33].

4. Conclusions

Beta thalassemia major patients have a significantly lower level of leptin and a significantly higher level of ferritin compared to the healthy controls. The increased ferritin level can have harmful consequences on fat cells, impairing their ability to produce leptin, and this leads to a reduction in leptin level.

Acknowledgments: We would like to acknowledge all those who have helped us in the preparation of this research. We extend our sincere thanks to the staff in Al-Karama Teaching Hospital, Hereditary Blood Disorder Center.

Conflicts of Interest: The authors confirm that there are no conflicts of interest.

References

[1] Angastiniotis M, Lobitz S.; " Thalassemias: an overview ". Int J Neonatal Screen., 5(1):1-11, 2019.

[2] Wafaa, F. A. M.; " The Beta-Thalassemia ". Sci. J. Med. Res., 1(1):24-30, 2017.

[3] Zhou, X.; Huang, L.; Wu, J.; Qu, Y.; Jiang, H.; Zhang, J.; and Lian, Q.; " Impaired bone marrow microenvironment and stem cells in transfusion-dependent beta-thalassemia ". Biomed Pharmacother., 146(1):112-117, 2022.

[4] Carsote, M.; Vasiliu, C.; Trandafir, A. I.; Albu, S. E., Dumitrascu, M. C.; Popa, A.; and Sandru, F.; " New entity—thalassemic endocrine disease: major beta-thalassemia and endocrine involvement ". Diagnostics., 12(8):1921-1949, 2022.

[5] Kattamis, A.; Forni, G. L.; Aydinok, Y.; and Viprakasit, V.; " Changing patterns in the epidemiology of β-thalassemia ". Eur J Haematol., 105(6):692-703, 2020.

[6] Chuncharunee, S.; Teawtrakul, N.; Siritanaratkul, N.; and Chueamuangphan, N.; " Review of disease-related complications and management in adult patients with thalassemia: A multi-center study in Thailand ". PLoS One., 14(3):21-33, 2019.

[7] Al-Bairmani, H. K.; Abdulhameed, Q. M.; and Alfartusie, F. S.; " Estimation of adenosine deaminase in adult iraqi patients with β-thalassemia ". J Glob Pharma Technol., 10(6):393-397, 2019.

[8] Recinella, L.; Orlando, G.; Ferrante, C.; Chiavaroli, A.; Brunetti, L.; and Leone, S.; " Adipokines: new potential therapeutic target for obesity and metabolic, rheumatic, and cardiovascular diseases ". Front Physiol., 11(1):57-88, 2020.

[9] Clemente-Suárez, V. J.; Redondo-Flórez, L.; Beltrán-Velasco, A. I.; Martín-Rodríguez, A.; Martínez-Guardado, I.; Navarro-Jiménez, E.; and Tornero-Aguilera, J. F.; " The role of adipokines in health and disease ". Biomedicines., 11(5):1290-1330, 2023.

[10] Giralt, M.; Cereijo, R.; and Villarroya, F.; " Adipokines and the endocrine role of adipose tissues ". Metab Control., 164(1): 265-282, 2016.

[11] Gautron L, Elmquist JK. " Sixteen years and counting: an update on leptin in energy balance ". J Clin Invest., 121(6):2087-2093, 2011.

[12] Kara H.; " Leptin, ghrelin, and other peptide hormones: an overview of their structures and functions ". Psychiatry Clin Psychopharmacol., 28(1):323-324, 2018.

[13] Ibraheam, Z. I.; " Correlation between Uric Acid and Liptin with Its Ratio in a Sample of Iraqi Patients with Diabetes Mellitus ", J. Al-Nahrain Uni. Sci. 14(3): 17-23, 2011.

[14] Friedman J.; " Leptin at 20: an overview ". J Endocrinol., 223(1):223-233, 2014.

[15] Friedman JM.; " Leptin and the endocrine control of energy balance ". Nat Metab., 1(8):754-764, 2019.

[16] Fantuzzi G,; Faggioni R.; " Leptin in the regulation of immunity, inflammation, and hematopoiesis ". J Leukoc Biol., 68(4):437-446, 2000.

[17] Manohar, S. M.; Shah, P.; and Nair, A.; " Flow cytometry: Principles, applications and recent advances ". Bioanalysis., 13(3):181-198, 2021.

[18] Yousefian, S.; Mirialiabad, G.; Saleh, R.; and Khedmati, M.; " Association of Body mass index and serum ferritin level in pediatrics with Beta-thalassemia major disease ". Iran J Pediatr Hematol Oncol., 12(1):34-40, 2022.

[19] Al-Naama LM.; Hassan MK.; Abdul Karim MM.; " Evaluation of serum leptin levels and growth in patients with β-thalassaemia major ". Anemia., 84(1):54-60, 2016.

[20] El-Deen, Z. M. M.; Ismail, A. M.; Meguid, M. M. A.; and Harb, M. T.; " Some endocrinal changes in children with β-thalassemia major ". Egypt J Haematol., 39(3):103-108, 2014.

[21] Shah, F. T.; Sayani, F.; Trompeter, S.; Drasar, E.; and Piga, A.; " Challenges of blood transfusions in β-thalassemia ". Blood Rev., 37(1):100-112, 2019.

[22] Rivella, S.; " Iron metabolism under conditions of ineffective erythropoiesis in β-thalassemia ". Blood, J Am Soc Hematol., 133(1):51-58, 2019.

[23] Khawaji, M. M.; Hazzazi, A. A.; Ageeli, M. H.; Mawkili, Y. H.; Darbashi, A. H., Kathiyah, A. M. A .A.; and Humedi, R. A.; " Clinical and hematological features among β-thalassemia major patients in Jazan region: A hospital-based study ". J Fam Med Prim care., 9(1):412-417, 2020.

[24] Tari, K.; Valizadeh Ardalan, P.; Abbas zadehdibavar, M.; Atashi, A.; Jalili, A.; and Gheidishahran, M.; " Thalassemia an update: molecular basis, clinical features and treatment ". Int J Biomed public Heal., 1(1):48-58, 2018.

[25] Arab-Zozani, M.; Kheyrandish, S.; Rastgar, A.; and Miri-Moghaddam, E.; " A systematic review and meta-analysis of stature growth

complications in ß-thalassemia major patients ". Ann Glob Heal., 87(1):1-17, 2021.
[26] Arshad, B.; Iqbal, T.; Jamil, A.; and Asan, I. J. H.; " Hematological and biochemical changes in male patients with thalassemia major ". Pak. j. life soc. Sci., 12(1):31-35, 2014.
[27] Roth, I. L.; Lachover, B.; Koren, G.; Levin, C.; Zalman, L.; and Koren, A.; " Detection of ß-thalassemia carriers by red cell parameters obtained from automatic counters using mathematical formulas ". Mediterr J Hematol Infect Dis., 10(1):201-210, 2018.
[28] AlAthari, R.A.; and Mahdi, W.T.; " Investigating Some Of Blood Parameters In Women With ß-Thalassemia ". In Journal of Physics: Conference Series., 1294(6):62-69, 2019.
[29] Sumneang, N.; Siri-Angkul, N.; Kumfu, S.; Chattipakorn, S. C.; and Chattipakorn, N., " The effects of iron overload on mitochondrial function, mitochondrial dynamics, and ferroptosis in cardiomyocytes ". Arch Biochem Biophys., 680(1):108-113, 2020.
[30] Hagag, A. A.; Ebtesam, R.; Maaly, M. M.; and Ibrahim, M. B.; " Study of serum leptin in children with beta thalassemia: Correlation with iron overload ". Med J Cairo Univ., 86(1):3037-3045, 2018.
[31] Ali, S.; Mumtaz, S.; Shakir, H. A.; Khan, M.; Tahir, H. M.; Mumtaz, S.; and Khan, M. A.; " Current status of beta thalassemia and its treatment strategies ". Mol Genet genomic Med., 9(12):1788-1801, 2021.
[32] Harbi, N. S.; Jawad, A. H.; and Alsalman, F. K.; " Evaluation of adipokines concentration in Iraqi patients with major and minor beta thalassemia ". Reports Biochem Mol Biol., 9(2):209-215, 2020.
[33] Al-Fatlawi, A. C. Y.; " Evaluation of leptin serum concentration in cases of blood transfusion dependent beta thalassemia and its relationship with thyroid dysfunction ". Biomedicine., 42(5):1029-1033, 2022.