Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2022.958

The Effect of Iron Overload on Erythrocyte Histological Composition, Insulin-like Growth Factor, and Liver Function in Beta Thalassemia Patients

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Submitted: 12 February 2022; Accepted: 11 April 2022; Published: 07 June 2022

ABSTRACT

This study is conducted in the College of Sciences / Department of Biology- University of Tikrit, in cooperation with the Thalassemia Center in Erbil Governorate, for the period from December 2021 - June 2022 to investigate the effect of iron overload on the histological composition of red blood cells and some physiological markers in beta thalassemia patients. A number of 120 individuals participated in this study, including 20 healthy individuals representing the control group of both sexes (11 males and 9 females) and 100 individual with beta thalassemia major who were undergoing treatment of both sexes (56 males and 44 females). Their average ages range between (3-36) years, and all disease cases have been diagnosed by specialized physicians. The results indicate through the colored blood slides that red blood cells appear generally hypochromic microcytic, as well as a discrepancy in the size and shape of red blood cells. Some of these red blood cells are large in size (macrocytosis), some in the form of a teardrop (ovalocyte), and others are target or stomatal or ghostly. It is also shown that there are significant differences in the shapes, sizes, and stains of RBCs in the blood smears of pathological cases (thalassemia) compared to the control group, but not significant when comparing males with females, except for the target cell, which has shown differences in this respect. As for the results of the analyzes of the chemical parameters GPT, GOT, ALP, the results show a significant increase in the activity of GOT, GPT and ALP enzyme in patients with beta thalassemia major compared to the control group, while there is a significant decrease in the level of IGF-1 concentration in patients with beta thalassemia major compared to the control group.

Keywords: *thalassemia, beta thalassemia major, insulin-like growth factor, ferritin, and blood smears*

INTRODUCTION

Thalassemia is a different group of genetically restricted hemoglobinopathies characterized by either absolute or reduced normal hemoglobin production, which leads to microcytic anemia of various degrees. Symptoms of the disease can be based on the type, and can vary from none to severe, as there is often moderate to severe anemia. Anemia causes fatigue, pale skin, bone problems, as well as extended spleen, deep urine, and tardy growth among children (Mahdi et al., 2018).

Thalassemia is described as a genetic disorder that exists in two main types: alpha thalassemia and beta thalassemia. The causative factors behind thalassemia are decreased production of the alpha / beta globin chain (Holm and Cherney, 2017). These types depend on the number of absent genes, four for alpha globin and two for beta globin. Moreover, the cause of erythropoiesis and hemolytic anemia is the accumulation of too many free alpha or beta globin chains, and their deposition on the membrane of red blood cells (Mahdi et al., 2018).Beta-thalassemia occurs with decreased or absent production of beta-globin subunits (Farid et al., 2019). Diagnosis is standardized by blood tests that include a complete blood count (CBC), special hemoglobin tests and genetic tests, and the diagnosis may be made during prenatal testing.Treatment of thalassemia depends on the type and severity of the disease. As for treatment of severe cases, it often includes regular blood transfusions, iron chelation and folic acid. Iron is chelated with deferoxamine or deferasirox. In some cases, bone marrow transplantation may be an option.Complications due to chronic hemolytic anemia can lead to an increase in iron intake and thus an increase in its quantity and deposition in the organs. This leads to major complications such as cardiac and endocrine disorders including diabetes, hypothyroidism, hypogonadism and infertility. Therefore, ineffective erythropoiesis (IE), iron overload (IOL), and chronic hemolytic anemia are the main complications of thalassemia, which is spread all over the world, especially in the Mediterranean region, Far East countries and Southeast Asia (Mahdi et al., 2018).Beta thalassemia consists of two main subtypes depending on the severity of the disease. Mutations of the beta-globin gene that negatively affect the production of the beta-globin subunit cause both. Heterozygotes with only one gene mutation cause beta-thalassemia minor, resulting in decreased production of beta-globin subunits. Although some patients may develop mild microcytic anemia, most are asymptomatic, and usually there is no evidence of hemolysis. The homozygotes with two genetic mutations cause beta-thalassemia major, which leads to the absence of production of beta-globin subunits. Deficiency of beta-globin results in accumulation of alpha-globin and alpha-tetrameric subunits that damage erythrocytes. Thus, ineffective erythropoiesis and extravascular hemolysis cause severe microcytic hypochromic anemia and patients with it require chronic blood transfusions (Farid et al., 2019).

MATERIAL AND METHODS OF WORK

Material

The blood samples are from patients with beta-thalassemia major who are registered at the Thalassemia Center in Erbil and are under continuing treatment. The number is (100) samples from both sexes (56 males and 44 females), with ages ranging from 3 years to 36 years. All the pathological conditions are diagnosed by specialized doctors. Sample collection began in December-June 2022, and patient information and data were recorded. In addition, there are samples of normal cases (control group), which include (20) blood samples of healthy people distributed according to sex and age group, with (11) males and (9) females. The ages of this group range between (9-32) years. Approved laboratory equipment and measuring kits are used to obtain the results.

Methods of Work

Blood Samples Collection and Preservation

Blood samples are collected without the use of a tourniquet. An amount of 5 ml of venous blood samples are collected from the patient before receiving a blood transfusion. The samples are placed in clean, dry plastic tubes with tight covers. The samples are distributed later according to the type of test, as the venous blood used in the complete blood tests is placed in tightly closed plastic tubes containing the anticoagulant EDTA tube. As for the rest of the tests, the blood is placed after separating the serum from it in plastic tubes with tight-fitting plain-tubes free of anticoagulants.

Preparation of Serum

The venous blood drawn from the patient is collected in clean, dry plastic tubes with tight

covers. These tubes are left for 5 minutes at room temperature and placed in a centrifuge for 10 minutes at a speed of 3000 rpm/min to obtain blood serum. Blood serum is withdrawn by single-use pipettes, placed in clean and sterile Eppendorf tubes free of any substance, and kept in a frozen state at a temperature of (-20) degrees Celsius until hormonal and enzymatic tests of the samples are conducted.

Estimation of Biochemical Markers

Biochemical markers (GOT, GPT, ALP) are measured by the Cobas integra 400 plus device, which operates according to four standard principles (Cobas, 2009): Absorbance photometry such as enzymes and substrates, turbidimetry such as drugs and proteins, fluorescence polarimetry such as therapeutic drugs and thyroid tests, and ion-selective electrode potentiometry such as lithium, sodium, potassium, and chlorine. Test tubes containing blood samples are placed in a centrifuge for 10 minutes at a speed of 3000 rpm/min to obtain serum, after which 500 microliters of serum are withdrawn by means of a pipette and placed in the sample cup in the Cobas integra 400 plus device. The data of the samples are entered with the analysis to be conducted (ALP,GPT,GOT) and the results are obtained after approximately 30 minutes.

Estimation of IGF-1 and Ferritin

The above analyzes are carried out using the Cobas e 411 device that works with ECL Electrochemiluminescence technology consisting of extreme accuracy and high sensitivity (Nawaz, 2011; cobas, 2009). The test tubes containing blood samples are placed in a centrifuge for 10 minutes at a speed of 3000 rpm. cycle/minute to obtain the serum. An amount of 500 microliters of serum is withdrawn by means of a pipette and placed in the designated sample cup in the device. After that, the data for the samples are entered with the required analyzes (IGF-1 and Ferritin) and the results are obtained after approximately 18 minutes.

Preparation and Analysis of Blood Smears

This is carried out by following these steps:

A drop of blood (about 5 microliters) is placed 1 cm away from one end of the glass slide.

The drop of blood is spread on the slide using another slide, which is placed at an angle of (30-40) degrees and in contact with the drop of blood, and then is withdrawn smoothly in one motion.

The slide is dried by waving it in the air.

The slide is then dipped in absolute ethyl alcohol for fixation, and left to air dry.

The smear is stained by immersing the slide for two minutes in Leishman's stain, then the stain is diluted with water by half, and the slide is left in it for (5-7) minutes, after which the slide is washed with water and left to dry.

The blood smear is examined using a compound light microscope with different magnification powers, then photographed with a digital camera Amscope mu300 connected to the microscope and a laptop computer. The magnification power of the objective lens and the magnification coefficient of the camera lens are calculated.

Statistical Analysis

The results are analyzed using the following statistical methods:

The results of the differences between males and females are calculated using the T-Test for blood cells.

The Mann-Witney test is used to compare the two groups of patients and the control of both males and females, with the statistical analysis carried out using the SPSS V26 program.

RESULTS AND DISCUSSION

A total of 100 patients with beta thalassemia major are studied, including 56 males and 44 females, whose ages range between (3-36) years, registered at the Thalassemia Center in Erbil and are still receiving treatment. All disease cases are diagnosed by specialized doctors. In addition, there are 20 healthy individuals regarded as a control group, consisting of 11 males and 9 females.

Diagnosis of Beta Thalassemia Major Cases

A smear of peripheral blood is taken on a clean glass slide for a selected sample of (14) patients, and two healthy samples representing the control group. The results are shown in Table (1).

Comparing males and females in each blood smear

The results shown in Table (1) indicate that there are significant differences between both males and females in the target cell of RBCs. This is in terms of the probability value (P-value), which amounts to (0.018) and which is less than (0.05). Here, the mean value of males is higher than the mean value for females. As for the rest of the tests, it is found that there are no significant differences between males and females in each of them, as indicated by the probability value that appears to be greater than (0.05).

TABLE 1: Cell shapes from a blood smear according to sex

Cells	Sex	No. of Samples	Medium	Standard Deviation	Standard Error	T-Value	P-Value
Microcyte	Male	10	27.69	8.28	2.6	-0.25	0.817
	Female	4	29.10	10.4	5.2		
Hipochromic	Male	10	16.17	5.30	1.7	-0.78	0.490
	Female	4	22.2	15.0	7.5		
Nucleated cell	Male	10	2.42	1.06	0.33	0.14	0.899
	Female	4	2.33	1.23	0.62		
Normoblast	Male	10	6.64	6.66	2.1	1.25	0.239
	Female	4	3.63	2.39	1.2		
Target cell	Male	10	5.11	3.41	1.1	2.78	**0.018
	Female	4	1.875	0.866	0.43		
Ghost cell	Male	10	5.46	3.99	1.3	0.56	0.590
	female	4	4.53	2.21	1.1		
Macrocyte	Male	10	12.17	5.92	1.9	0.06	0.953
	female	4	11.8	10.2	5.1		
Roulex	Male	10	2.18	1.02	0.32	-0.51	0.643
	female	4	2.73	2.02	1.0		
Poikilocyte	Male	10	2.46	1.27	0.40	0.33	0.745
	female	4	2.30	0.523	0.26		
RBC with Hb fraction	Male	10	2.82	1.46	0.46	-0.20	0.845
	female	4	2.950	0.896	0.45		
Ovalocyte	Male	10	3.67	2.62	0.83	1.18	0.263
	female	4	2.575	0.830	0.42		
Orocyte	Male	10	1.79	1.35	0.43	-0.91	0.415
	female	4	2.80	2.05	1.0		
Micro normoblast	Male	10	2.02	1.81	0.57	-0.54	0.628
	female	4	3.28	4.52	2.3		
Spiny cell	Male	10	3.38	3.36	1.1	0.69	0.508
	female	4	2.38	1.99	1.0		
RBC with incomplete Hb ring	Male	10	5.14	3.20	1.0	0.69	0.508
	female	4	5.93	3.79	1.9	-0.37	0.733

Two – Sample T-Test

T – test of difference = 0 Vs. T – test of difference ≠ 0

** The presence of a significant difference

Red blood cells are surrounded by a plasma membrane consisting of lipids, proteins and carbohydrates. Preserving the shape of the red blood cell is one of the most important functions of this membrane. However, some changes occur in its chemical composition during the passage of red blood cells in the blood, which leads to an imbalance in the membrane, and this affects the shape of the cell and its functions and makes it vulnerable to decomposition and breakage during its passage through the blood in the event of infection with some diseases (Ibrahim and Rasheed,2017). The results of pathological cases (thalassemia) have shown a significant difference in the appearance of red blood cells as in Figure (3) compared to the control group in Figures (1) and (2), as most of the abnormal forms of red blood cells are clearly present in patients' blood smears. The appearance of variation in the shapes, sizes, and colors of RBCs, noting the presence of normal ones in normocyte size and normochromic color, represents the remnants of transfused blood for patients (donor blood). It is also observed that RBCs contain dispersed hemoglobin (Hb fraction), which appears in the form of dark dots called basophilic stippling, illustrated by Figure (13). Another difference is observed in the shape of the RBCs, represented by the accumulation of hemoglobin in the center and periphery of the corpuscle with a pale area separating them. This condition is known as the target cell, as the stain is concentrated in the center and the edge, so it resembles the target at which the arrow is thrown, as shown in Figures (3) and (6). These results are consistent with what is mentioned by Walaa (2000) in the appearence in patients who underwent splenectomy. The results also show crescent cells, ovalocytes, and oral cells that contain a two-sided concave shape in its center, thus resembling a mouth. There are also microcyte cells, which is a condition in which the size of red blood cells is less than their normal size, and this condition occurs due to a deficiency in the materials needed by the process of forming red blood cells, erythropoiesis, such as vitamins, minerals, amino acids, and hormones. In addition there are cells of varying shape and size, poikilocyte, which is known as polycythemia of varying shape and size, occurring due to a defect in the process of formation of red blood cells, and ghost cells in which hemoglobin concentration appears less than its normal concentration, so the red blood cell appears pale in color. Moreover, there are red blood cells with incomplete Hb ring as shown in the figures below. Furthermore, the results of blood smears have resulted in red blood cells characterized by their large size, lack of macrocyte color, and hypochromic cells. This occurs due to a defect in the process of forming red blood cells and a lack of materials needed by the erythropoiesis process, such as vitamin B12, folic acid, and others. Other spiny cells have also appeared as in Figs. (6), (7), and (9), as well as immature red blood cells called nucleated RBC. This cell does not appear in the peripheral blood of healthy people, but its presence is normal only in the bone marrow. The rouleaux formation phenomenon is also observed, which is a state of adhesion of red blood cells, forming what looks like a compact row superimposed on top of each other as in Figures (12) and (13). The abnormal shape of the red blood cells indicates the possibility of a thalassemia effect that causes these blood cells to be rapidly destroyed, which requires patients to undergo regular blood transfusions to maintain them (Tyas et al., 2020). There are also other diseases that cause morphological and voluminous changes in the red blood cells, for example, Macrocyteanemia, sickle cell anemia, as well as the disease known as Hereditary Spherocyteosis, which is a genetic disease in which the red blood cell is spherical and thus dissolves easily during circulation in the blood (Ballas, 1990). These cases have been studied by many researchers, and they found that there are changes in the components of the plasma membrane that lead to changes in the composition of the membrane and thus a change in the external shape of the red blood cell (Farah et al., 2013). The results of the study also show the existence of the phenomenon of arrays or what is called rouleaux, which is a state of adhesion and superposition of red blood cells on top of each other, as their surface in normal conditions possesses a negative electric charge attributed to glycoproteins. This causes the phenomenon of interaction and adhesion of red blood cells to each other and to other vital surfaces as a result of their surface viscosity and

high density, but quickly return to separate from each other thanks to the flexibility of these globules during their stampede through the blood cycle. Infections, connective tissue disorders, cancer, and diabetes mellitus are among the causes of plaque formation (Ibrahim and Rasheed, 2017). As for the appearance of red blood cells of variable size in the results of this study, it is attributed either to a deficiency in the materials needed for the formation of red blood cells, erythropoiesis, such as vitamins, minerals, amino acids and hormones, or to a decrease in the activity of the Na-K-ATPase enzyme pump in its membranes. This leads to an increase in the size of the blood cells and their osmotic fragility, as well as a decrease in their filterability in reducing the entry of potassium ions K from outside the cell into the inside and vice versa for sodium ions Na. This causes an increase in the concentration of K outside the cell and a decrease in the concentration of Na, which leads to disturbance in the capillary blood circulation, which can cause the decomposition of some red blood cells and the occurrence of anemia that arises when the number of healthy RBCs in the body is insufficient or less than the normal number (Farzana et al., 2006; Alkanaani, et al.,2020).

The plasma membrane contains many proteins of different molecular weights. These proteins are located intertwined within the two layers of lipids and partially surrounding them in the plasma membrane, which plays an essential and important role in the structural construction of the membrane and the preservation of its chemical and physical properties. Thus, any deficiency in these proteins leads to a defect in membrane functions (Ballas, 1990). One study found that the lack of Band 4.2 protein in Japanese people caused an imbalance in the amount of proteins, which led to hemolytic anemia (Rybicki et al., 1993). Also, proteins that interfere with fats can be affected by the presence of fats in large quantities in the membrane, as the increase in the percentage of fats puts pressure on these proteins and thus affects the overlap between them, and this imbalance in turn affects the composition and properties of the membrane (Needham and Nunn, 1990).

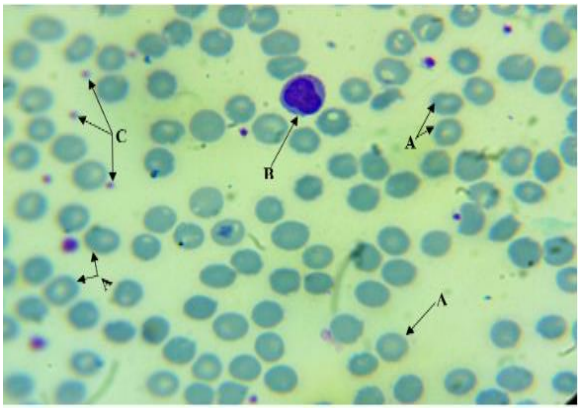

FIGURE 1: A micrograph of a peripheral blood smear from a control group, showing(A)normocytic red blood cells with normochromic stain, as well as (B) a normal appearance of a monocyte and (C)platelets. (Lishman colored, x100).

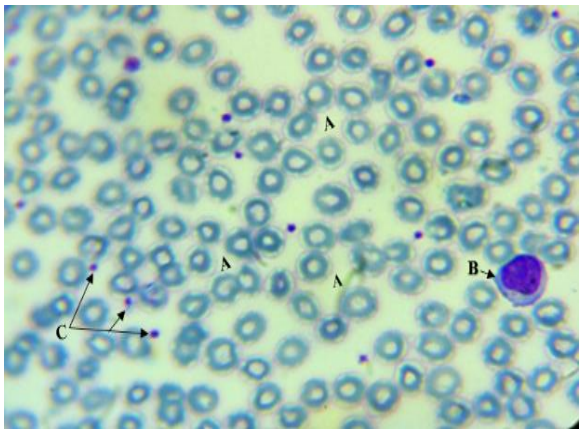

FIGURE 2: A micrograph of a peripheral blood smear from a control group, showing (A) normal red blood cells in shape, size, and stain, as well as (B) a normal appearance of a lymphocyte and (C) platelets. (Lishman colored, x100).

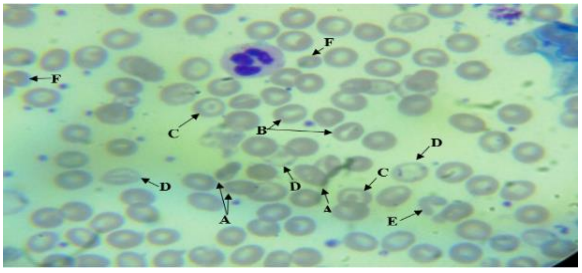

FIGURE 3: A micrograph of a peripheral blood smear of a patient with treated beta thalassemia major, showing (A) red blood cells: ovalocyte, (B)orocyte, (C) target cell, (D) ghost cell, (E) crescent cell, and (F) small microcyte. (Lishman colored, x100).

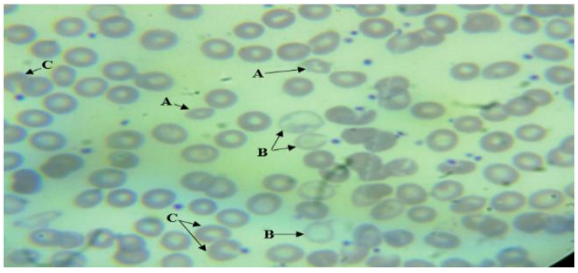

FIGURE 4: A microscopic image of a peripheral blood smear of a patient with treated beta thalassemia major, showing (A)red blood cells: small, (B) ghosty, and (C) crescent. (Lishman colored, x100).

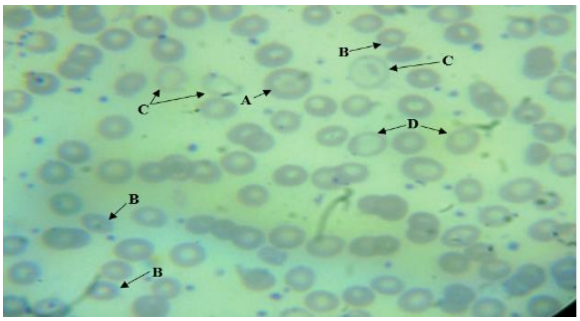

FIGURE 5: A micrograph of a peripheral blood smear of a patient with treated beta thalassemia major, showing (A) red blood cells: macrocyte, (B) small, (C) ghostly, and (D) hypochromic. (Lishman colored, x100).

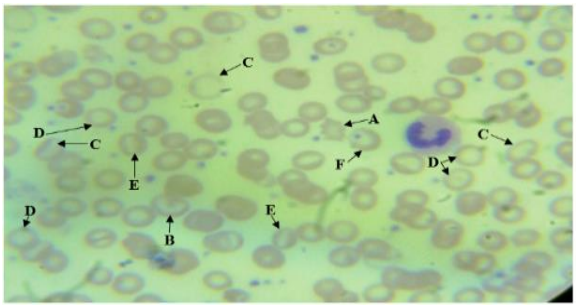

FIGURE 6: A microscopic image of a peripheral blood smear of a patient with treated beta thalassemia major, showing (A) red blood cells: spiny cell, (B) target, (C) ghostly, (D) hypochromic, (E)oral, and (F) incomplete hemoglobin loop. (Lishman colored, x100

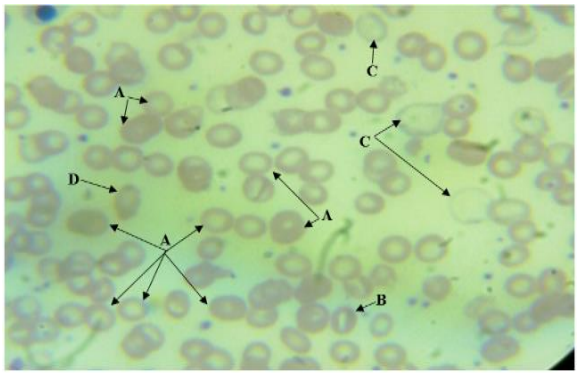

FIGURE 7: A micrograph of a peripheral blood smear of a patient with treated beta thalassemia major, showing (A) red blood cells: anisochromic, (B) oral, (C) ghostly (C), and (D) spiny. (Lishman colored, x100).

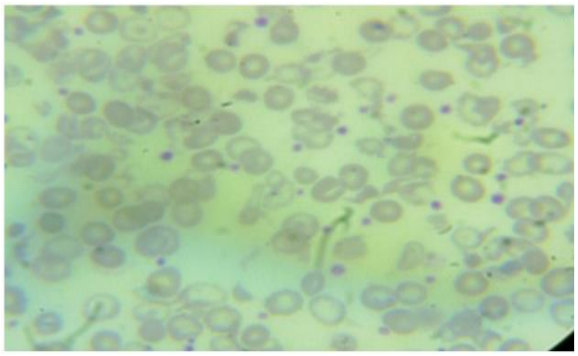

FIGURE 8: A micrograph of a peripheral blood smear from a patient with beta thalassemia major, showing acute poikilocyte necrosis of erythrocytes (disparity in shape and size) and pigment abnormalities throughout the smear. (Lishman colored, x100).

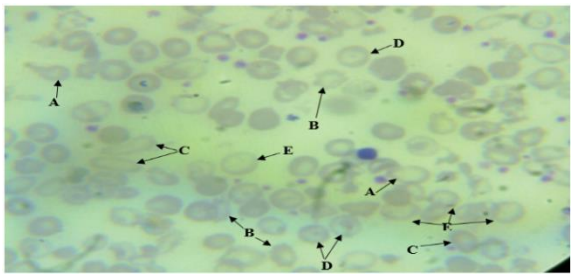

FIGURE 9: A micrograph of a peripheral blood smear of a patient with treated beta thalassemia major, showing (A) red blood cells: teardrop cell, (B) spiny cell , (C) oval (D) target , (E) ghostly. (Lishman colored, x100).

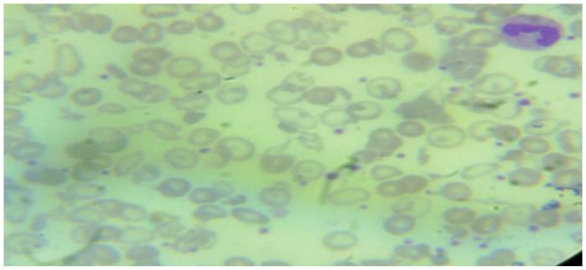

FIGURE 10: A microscopic image of a peripheral blood smear of a patient with treated beta thalassemia major, in which red blood cells of different sizes with hypochromic anisocytosis appear throughout the blood smear. (Lishman colored, x100).

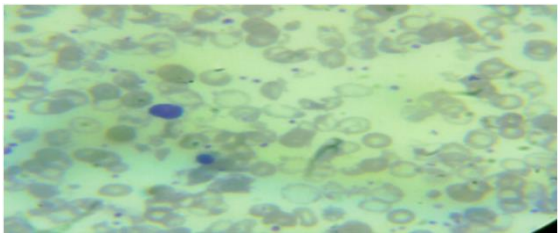

FIGURE 11: A microscopic image of a peripheral blood smear of a patient with beta thalassemia major, in which red blood cells of varying size, shape, and pigmentation appear sharply throughout the blood smear. (Lishman colored, x100).

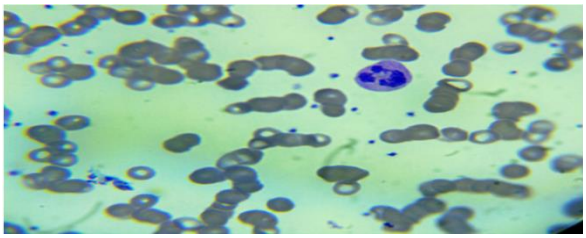

FIGURE 12: A microscopic image of a peripheral blood smear of a patient with treated beta thalassemia major, showing the occurrence of rouleaux phenomenon (adherence of red blood cells in a row-like manner) in the entire blood smear. (Lishman colored, x100).

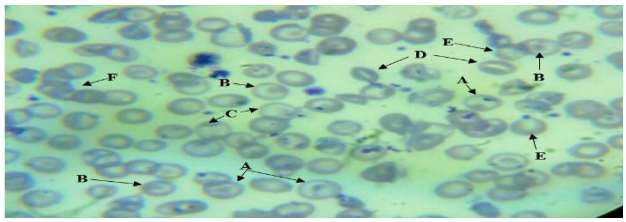

FIGURE 13: Micrograph of a peripheral blood smear from a patient with treated beta thalassemia major, showing (A) red blood cells: dispersed (fractionated) hemoglobin Hb fraction, (B) target, (C) ghostly, (D) stomatal, (E) incomplete hemoglobin ring, as well as (F) the occurrence of the phenomenon of rouleaux. (Lishman colored, x100).

Immunological variables for the two groups (patients and control) according to sex

IGF-1 Concentration

The results of the current study, as shown in Table (2) and Figure (14), show a significant decrease (P≤0.05) in the level of IGF-1 concentration (69.76±99.16) ،69.04±95.38) in patients with beta thalassemia major and undergoing treatment, males and females, respectively. , when compared with the control group of males and females ،(41.5±216.09) (47.67±225.56), respectively.

TABLE 2: Concentration of immunological variables for the two groups (patients and control) according to sex

Standards	Sex	Patients Group	Control Group	P- Value
IGF-1	Male	95.38 ±69.04	216.09 ±41.5	0.000
	Female	99.16 ±69.76	225.56±47.67	0.000
Ferretin	Male	1660.88±543.4	37.51 ±19.1	0.000
	Female	1742.46±505.22	11.90±0.65	0.000

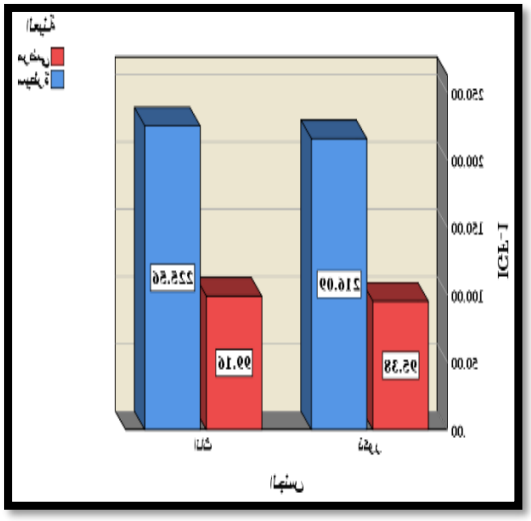

FIGURE 14: IGF-1 concentrations for the two groups (patients and control) according to sex

Ferritin Concentration

The results show that there is a significant increase (P≤0.05) in ferritin concentration (543.4±1660.88) and (505.22±1742.46) in patients with beta thalassemia major who are undergoing treatment, males and females, respectively, when compared with the control group of males and females (19.1±37.51) and . (0.65±11.90), respectively, as shown in Table (2) and Figure (15).

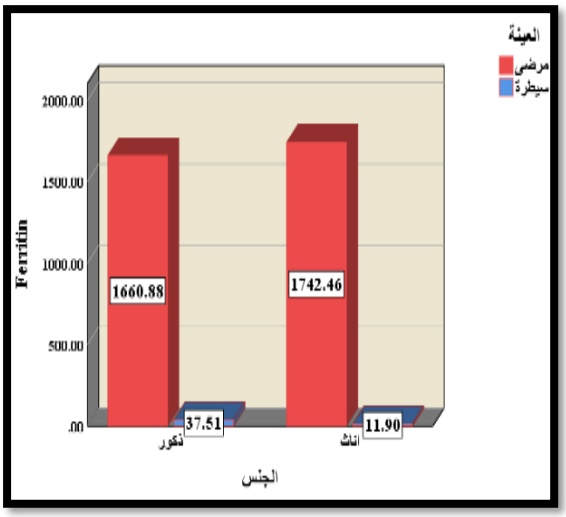

FIGURE 15: Ferritin concentration for the two groups (patients and control) according to sex

The decrease in the concentration of IGF-1 may be mainly due to the inability to secrete growth hormone successfully and this decrease contributes to the removal of bone minerals in thalassemia patients, in addition to the disruption of the endocrine system due to the excess iron that occurs in many body systems (Franchini and Massimo, 2006; Mukhopadhyay et.al., 2015). Low IGF-1 levels without GH deficiency indicate a possible association with chronic anemia, malnutrition, or impaired growth hormone secretion (Isik et al., 2014). IGF-1 plays an important role in bone remodeling, and low levels in the blood reduces the proliferation of osteoblasts and the formation of extracellular matrix of the bone, and also reduces the activation of osteoclasts. A correlation is found between bone mineral density in the lumbar spine and IGF-1 concentration (Morabito et al., 2016).The results of the current study agree with the study of Gaudio et al. (2019), that there is a decrease in the level of IGF-1 concentration in patients with beta thalassemia major compared to the control group, and that there is a significant relationship between IGF-1 and osteocalcin, which is a marker of bone formation and BMD values. . The results of the current study also agree with the study by Al-Naama et al. (2020) in that the concentration of IGF-1 is low in more than half of the patients, and this result is much lower than that found in the control group. Among patients with short stature, 92% of them have a decrease in IGF-1 concentration, and even in patients of normal stature, the average level of IGF-1 is significantly lower compared to the control group. These results are similar to what is reported by Riza et al. (2019) (Khaleel et al.,2019). The results of this study are also similar to what is found by Jwaid and Gata (2020) and Sharif et. al. (2021) in that the ferritin concentration level has increased in patients compared to the control group. Ferritin testing is useful for monitoring treatment in patients who have not yet experienced a significant increase in stored iron. The iron excess observed in thalassemia patients may be due to chronic transfusion and hypercalcemia (Kassab-Chekir et al., 2003). Iron overload in beta-thalassemia patients can lead to intestinal iron absorption and an abnormal iron molecular shape. Elevated iron also plays a major role in the oxidation of cell membranes, which can be one of the main pathways in the removal of red blood cells. Increased intestinal iron absorption, frequent blood transfusions, peripheral hemolysis, and ineffective erythropoiesis are associated with the accumulation of iron within various organs such as the liver, kidneys, heart, and endocrine glands (Shanaki et al., 2016; Rasool et al., 2016). This iron accumulation leads to cirrhosis, endocrine abnormalities and heart disease (Origa, 2017).

Liver enzymes for both patients and control groups according to sex

GOT Enzyme Effectiveness

The results of the current study, as shown in Table (3) and Figure (16), show a significant increase ($P \leq 0.05$) in the effectiveness of the GOT enzyme by (3.53±34.75) (2.63±37.48) in patients with beta thalassemia major and undergoing treatment, males and females, respectively, when compared to the control group of males and females ،(3.424±29.93) (7.42±20.84), respectively.

TABLE 3: The effectiveness of liver enzymes for the patients group compared with the control group according to sex

Standards	Sex	Patients Group	Control Group	P- Value
GOT	Male	37.48 2.63±	29.933.424 ±	0.034
	Female	34.753.53±	20.847.42±	0.017
GPT	Male	18.90 ±5.22	15.73 ±3.13	0.040
	Female	16.78±2.99	13.50±10.12	0.039
ALP	Male	149.23±76.70	35.62 ±4.9	0.000
	Female	108.12±42.31	27.7±5.7	0.000

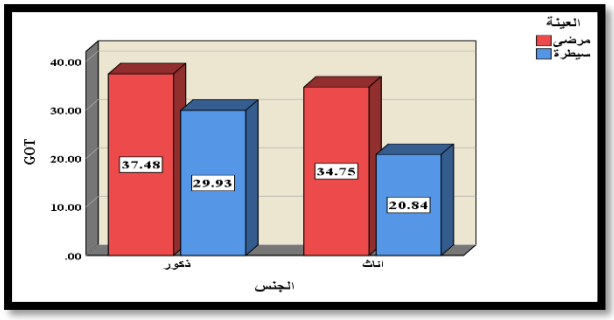

FIGURE 16: The effectiveness of GOT enzyme for the patients group compared with the control group according to sex

GPT Enzyme Effectiveness

The results of the current study, as shown in Table (3) and Figure (17), show a significant increase (P≤0.05) in the effectiveness of GPT enzyme in patients with beta thalassemia major undergoing treatment, males and females (5.22±18.26) and(2.99±16.60) , respectively compared with the control group of males and females 3.13±15.73) and (10.12±13.50) , respectively.

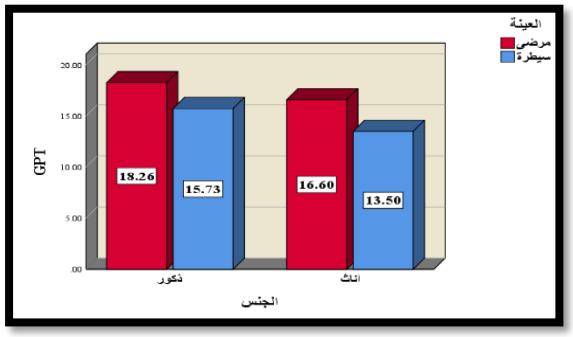

FIGURE 17: The effectiveness of GPT enzyme for the patients group compared with the control group according to sex

ALP Enzyme Effectiveness

The results of the current study, as shown in Table (3) and Figure (18), show a significant increase (P≤0.05) in the effectiveness of ALP enzyme (76.70±149.23) and (42.31±108.12) in patients with beta thalassemia major and undergoing treatment, males and females, respectively when compared to the control group of males and females (4.9±35.62) and (5.7±27.70), respectively.

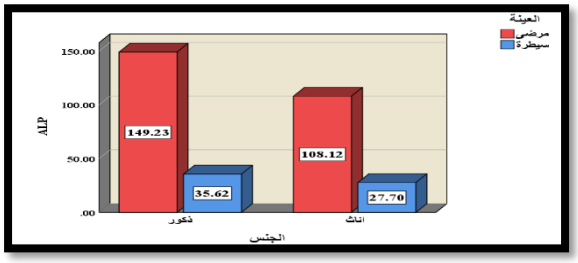

FIGURE 18: The effectiveness of ALP enzyme for the patients group compared with the control group according to sex

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Tietz (1986) indicates that GPT and GOT are important enzymes in the diagnosis of diseases, and that their concentration in the heart, liver, kidney and skeletal muscles is more than their concentration in blood serum. Thus, any damage to these tissues leads to a clear increase in their concentration in blood serum . The results of this study are consistent with Ali et al. (2020), as the activity of GOT and GPT enzymes increased in patients group compared to control group, and the main reason for this increase is the increase in iron. The results of this study are also consistent with what Abdulla (2018) mentioned about the presence of an increase in the level of activity of GOT enzymes and GPT in patients group compared to control group, as frequent blood transfusions cause an increase in iron load in thalassemia patients without chelation therapy, which leads to many complications such as enlargement of the spleen and the effect on liver functions, thus increasing GPT and GOT (Fadhil et al., 2015). Osteoporosis is also one of the most important complications of thalassemia, and the increase in ALP enzyme may be attributed to the fact that most of the activity of this enzyme comes from bone tissue. Thalassemia patients suffer from the dissolution of this tissue, which leads to leakage of this enzyme into the blood circulation, and then an increase in its activity (Jabbar and Mahdi, 2022). ALP enzyme is found in the liver and bone marrow. The bulk of it in blood serum comes from these two tissues (liver - bone marrow) and is secreted into the blood circulation. Therefore, the high activity of this enzyme is often due to the pathological conditions of these two tissues, or perhaps due to the effect of iron on hepatocytes, as an increase in the concentration of iron may destroy liver cells, which leads to the transfer of this enzyme in large quantities to the bloodstream and thus increase its effectiveness compared to healthy people. It is expected that the level of ALP increases in obstructive jaundice is more than hepatic jaundice resulting from hepatitis C infection. Inflammatory liver diseases, fibrosis, biliary strictures, cholecystitis, bile duct infection and hepatic tumors can also lead to elevated liver enzymes and alkaline phosphatase (Muhammad et al., 2013). The results of the current study agree with what is stated by Jwaid and Gata (2020) that the activity of ALP enzyme increases in the patients group compared to the control group. These results also agree with what is stated by Ali et al. (2020) in explaining the rise of this enzyme in patients with beta thalassemia major, which may be a result of an enlarged spleen disorder actually diagnosed by doctors in thalassemia patients.

The Correlation Analysis

The results of the correlation analysis between the variables studied for the group of male patients shown in Table (4) show that there is an inverse and significant correlation between ferritin and IGF-1, while there is a direct and significant correlation between ferritin and GOT, GPT and ALP.

TABLE 4: Correlation between ferritin and some tests for males

Correlation		
		Ferritin
IGF-1	Correlation Coefficient	-.603**
	P- Value	.000
GOT	Correlation Coefficient	.376**
	P- Value	.003
GPT	Correlation Coefficient	.681**
	P- Value	.000
ALP	Correlation Coefficient	.368**
	P- Value	.002

The results of the correlation analysis between the variables studied for the group of female patients shown in Table (5) also show that there is an inverse and significant correlation between

ferritin and IGF-1, while there is a direct and significant correlation between ferritin and GOT, GPT and ALP.

TABLE 5: Correlation between ferritin and some tests for females

Correlation		
		Ferritin
IGF-1	Correlation Coefficient	-.464**
	P- Value	.000
GOT	Correlation Coefficient	.405**
	P- Value	.006
GPT	Correlation Coefficient	.539**
	P- Value	.002
ALP	Correlation Coefficient	.440**
	P- Value	.001

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