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Assessment of Interleukin 1-β, Interleukins -6 and Some Biochemical Parameters in a Sample of Iraqi Patients with β –Thalassemia Major

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Abstract

Sixty patients (male and female) with β-thalassemia major and thirty healthy subjects were enrolled in this study during their attendance at "Abin AL-Baladi Hospital in Baghdad" from September 2022 to January 2023. All patients and healthy subjects ranged in age from 15 to 30 years, and we collected the necessary data with their consent, adhering to the College of Science's ethics (CSEC/0922/0104 in 2022/9/28). Venous blood was collected and divided into two parts: the first part for the determination of CBC, and the second part was allowed to coagulate for serum separation to measure the level of C-reactive protein, ferritin, and pro-inflammatory cytokines like interleukin-1β and interleukin-6 by using the ELISA Kit. The results showed a highly significant ($p \leq 0.01$) decrease in RBC count and hemoglobin (3.03 ±0.06 106/µL and 8.01±0.17 Hg/dl) respectively in β-thalassemia patients in compare with control group (4.77±0.07 106/µL and 14.06 ±0.24 Hg/dL) respectively, while there was a highly significant ($p \leq 0.01$) increase in the platelets count (384.59 ±21.67 10^3/µL) in patients compared with control (224.06 ±6.23 10^3/µL); furthermore, ferritin and CRP showed highly significant ($p \leq 0.01$) increase (4910.15±202.67 ng/ml, and 3.02 ±0.08 µg/ml) respectively in patients compared with control (284.91±16.78 ng/ml, and 1.178 ±0.03 µg/ml) respectively. Also, there was a highly significant ($p \leq 0.01$) increase in IL-1β and IL-6, which were 284.19 ±4.29 pg/ml and 27.92±0.97 pg/ml, respectively, in patients in comparison with control (142.09 ±0.70 pg/ml and 11.43 ±0.32 pg/ml), respectively. From the above findings, it can be concluded that proinflammatory cytokines have a significant impact on the progression and pathology of the disease.

Keyword: β-thalassemia major, ferritin, interleukin -1β, interleukin-6.

1. Introduction

Thalassemia is associated with the majority of monogenic illnesses, which are hereditary hemoglobinopathies that are characterized by inadequate erythropoiesis, hemolysis, and accelerated red blood cell turnover. The genetic disorder known as thalassemia is believed to decrease the production of the alpha or beta chains of hemoglobin [1]. If the body fails to produce either of these two proteins sufficiently, it disrupts the proper formation of red blood cells, preventing them from carrying sufficient oxygen; this leads to anemia that persists

throughout a person's life, starting in early childhood [2]. More than 150 distinct mutations on chromosomes 11 and 16 have been discovered to be responsible for the cases of α- and β-thalassemia, respectively. Because people with thalassemia don't make enough β-globulin, erythrocyte precursors make too many α-globulin chains.

This hurts the bone marrow and the blood flow to the body's surfaces [3]. Beta-thalassemia major is a genetic hemoglobin synthesis disease that causes severe anemia in homozygous individuals. The homozygous illness was previously known to be prevalent in the Middle East and Mediterranean nations, but migration has altered its geographic distribution and made it a global health issue [4]. The disease is associated with toxemia, increased bone marrow space, splenomegaly, extreme anemia, jaundice, and cardiomegaly.

These signs start to show between two and four months of age [5]. It comes in three main forms: "β-thalassemia major (TM)", also called "Cooley's anemia" and "Mediterranean anemia," "β-thalassemia intermediate (TI)", and "β-thalassemia minor", also called "heterozygous β-thalassemia," "β-thalassemia carrier," and "β-thalassemia trait" [6]. Beta-thalassemia results from a relative abundance of α-chains caused by decreased β-globin chain formation. The damage that free α-globin does to red blood cell membranes causes reactive oxygen species to form precipitate, which leads to hemolysis and abnormal erythroid maturation [7].

"Beta-thalassemia major, also called Cooley's anemia, is a very bad type of thalassemia that can happen to people who are homozygous ($^{2}+/^{2}0$, $^{2}0/^{2}0$) or compound heterozygous ($^{2}+/^{2}+$) for much worse mutations in the chain" [8]. The majority of patients receive recurrent blood transfusions instead of stem cell transplantation, which is an effective but not always practical therapy option[9].

The liver, spleen, myocardium, placenta, and other organs produce ferritin, a huge macromolecule with a molecular weight of 450 kDa, which is crucial for iron storage. Since serum ferritin measurement is most commonly used in the differential diagnosis of anemia because ferritin is a sensitive sign of iron shortage, iron excess (hemochromatosis or haemosiderosis), infection or inflammation, neurological illnesses, cancers, and liver tissue damage can all result in a rise in ferritin levels [10]. Ferritins have special chemical and physical characteristics. In addition to being stable in a variety of denaturants like urea or guanidinium chloride, ferritin can withstand extreme temperatures of up to 75 °C for a period of time. The abundance of salt bridges and hydrogen bonds between its subunits contribute to ferritin's unique properties [11]. Furthermore, ferritin is the primary protein that stores iron in the body. These proteins control the production of mRNA by acting as iron regulatory proteins. It plays a crucial role in maintaining iron homeostasis because of its ability to bind to and sequester intracellular iron [12].

The liver creates CRP, a pentameric protein whose concentration rises in response to inflammation. IL-6 primarily triggers the gene responsible for CRP transcription during the acute phase of an inflammatory or viral event [13]. C-reactive proteins provide the first line of defense against infections. Even though they are structurally different, CRP and immunoglobulin (Ig) molecules share many functional traits. The binding specificities and the site of synthesis of CRP, which give rise to a new superfamily of proteins, are additional distinguishing features. The quantitative measurement of CRP levels in serum is widely used by clinical practitioners as a sensitive indicator of inflammation. In addition to its involvement in removing germs and damaged or dying cells, CRP may also perform more intricate immunomodulatory tasks [14]. In β-thalassemia, CRP is primarily employed as a marker of infection and inflammation. CRP

readings can be measured and plotted to help track the progression of a disease or evaluate the efficacy of treatment [15].

Pro-inflammatory cytokines are small signaling proteins that are necessary for beginning and promoting inflammatory reactions in diseases, and they are up-regulated during inflammation. [16]. Cytokines regulate the balance between innate and adaptive immune responses. Proinflammatory and anti-inflammatory cytokines, including IL-6, IL-1, TNF-β, and IL-10, have a significant impact on the immune response. An imbalance in their production may result in immunological dysfunction, encouraging the genesis of inflammatory disorders [17].

Interleukin-1β is one of the pro-inflammatory cytokines thought to be involved in the evolution of inflammatory disorders such as sepsis, diabetic nephropathy, cardiovascular disease, and autoimmune disease [18]. Interleukin-1β is made by different types of cells, like B-cells, macrophages, and fibroblasts. It has two structurally different forms, IL-1α and IL-1β, which each have their functions [19]. A higher level of IL-1 in the blood of people with β-thalassemia means that more cells are dying, which makes erythropoiesis less effective in these people [20].

A diverse range of cells, including smooth muscle cells, mononuclear macrophages, T helper 2 cells, vascular endothelial cells, fibroblasts, and B cells, produce interleukin-6, a 186 amino acid glycosylated protein [21]. This soluble cytokine influences the immune response, inflammation, and hematopoiesis in numerous ways. Released in response to infections and tissue damage, it serves as a key mediator of fever and the acute phase of the immunological reaction, boosting the immune response [22]. It is well known that IL-6 plays a significant role in the proinflammatory response, and that its high serum level may affect the pathophysiology of β-thalassemia [23].

2. Materials and methods

Sixty patients (male and female) with β-thalassemia and thirty healthy subjects were interested in this study because of their presence at "Abin Al-Baladi Hospital in Baghdad." We conducted the research from September 2022 to January 2023. Despite having thalassemia major for varying lengths of time, all patients received normal treatment.

All patients ranged in age from 15 to 30 years, and we collected the necessary data from them with their consent, adhering to the Ethics Code CSEC/0922/0104 of a science college in 2022/9/28. Clinicians consistently checked each patient once or twice every month; they received erythrocyte transfusions on a regular basis. All patients had similar transfusion characteristics and durations.

We used a five-ml disposable syringe to collect venous blood from all the patients through vein punctures, dividing it into two parts: we transferred the first part to an EDTA tube for CBC determination, and then moved the second part to a gel tube to allow it to coagulate. We spun the serum at 3000 rpm for 10 minutes to determine the levels of CRP and ferritin. The kit includes an enzyme-linked immunosorbent assay (ELISA) for measuring pro-inflammatory cytokines such as IL-1 and IL-6. This method is "based on biotin double antibody sandwich technology." We stored the samples at 20 °C prior to analysis.

3. Results and Discussion

The results of this study showed that there is no significant (P>0.01) difference in age between patients with β-thalassemia major (19.38 ±0.85) years and controls (21.70 ±0.77) years. The study found that people with b-thalassemia had significantly lower mean RBC and Hb levels (4.77 ±0.07 106/µL and 14.06 ±0.24 g/dL) than healthy controls (P≤0.01). However, **Table 1** shows that the mean PLT levels were significantly higher (P≤0.01) in people with β-thalassemia (384.59 ±21.67 103/µL) compared to healthy people (224.06 ±6.23 103/µL).

Table 1. Age and blood parameters value in β-thalassemia major patients and control**.**

Group	Mean ± SE			
	Age (year)	**RBC (10^6/µL)**	**Hb (g/dL)**	**PLT ($10^{\wedge}3$/µL)**
Patients	19.38 ±0.85	3.03 ±0.06	8.01 ±0.17	384.59 ±21.67
Control	21.70 ±0.77	4.77 ±0.07	14.06 ±0.24	224.06 ±6.23
T-test	2.640 **	0.194 **	0.603 **	61.716 **
P-value	NS	0.0001	0.0001	0.0001

** (p≤0.01) , NS

This study showed that there was no significant difference between the selected age groups. The explanation for this result may be due to the selection of samples.

In the past three years, there has been a significant change in the normal course of β-thalassemia, and a sizable community of patients who are over 40 years old is now present [24]. Academic scholars have already expressed interest in beta-thalassemia, one of Asia's most common genetic diseases and the majority of the world. Thalassemia syndromes are a group of serious genetic disorders that develop in children and adolescents when one of the thalassemia or hemoglobin Lepore genes is homozygous [25].

The quantity of red blood cells was low in the present study, and the Hb result was comparable to that of [26], who discovered that the BTM group's mean values for all RBC parameters (MCV, MCHC, MCH, Hct, and Hb) were lower than those of the control group.

Noteworthy is that [27] discovered that RBC in thalassemia patients exhibits a significant deficiency in hemoglobin chains, either being absent of hemoglobin β or carrying beta chains but lacking in +β production. This often occurs during the erythropoiesis formation stage, which encompasses numerous stages, the most notable of which is differentiation. Erythropoiesis is the process that creates RBC from the hematopoiesis stem cell in the bone marrow.

Moreover, the sovereignty will be for α-type chains in both circumstances (β0 or β+), meaning that productivity will favor alpha over beta. Red blood cells will undoubtedly acquire these excess alpha chains, resulting in uneven red blood cells that are unable to transport a lot of oxygen throughout the body [28]. One of the study's most significant findings is that hemoglobin chain imbalances cause red blood cells to die quickly—120 days sooner than they would normally do. This leaves the bone marrow unable to replace the lost RBC, which means that compared to newly formed cells, the damaged cyst of red blood cells is higher. We would have misshaped bones as a result of the enhanced erythropoiesis processes and osteoporosis brought on by the double-reactive pellets' depletion of spinal cord components [29].

In addition, the current study is compatible with [30]. Patients with thalassemia have considerably more platelets (PLT) than individuals without thalassemia. The potential causes of these patients' elevated platelet counts are the expansion and maturation of marrow mononuclear cells to produce colony-forming unit megakaryocytes (CFUMeg).

As shown in **Table 2,** ferritin and CRP showed a highly (P≤0.01) significant increase in patients with β-thalassemia (4910.15 ±202.67 ng/ml and 3.02 ±0.08 µg/ml), respectively, as compared with control (284.91 ±16.78 ng/ml and 1.178 ±0.03 µg/ml), respectively.

Table 2. Level of Ferritin and CRP in β- thalassemia major patients and control.

Group	Mean ± SE	
	Ferritin (ng/ml)	**CRP (µg/ml)**
Patients	4910.15 ±202.67	3.02 ±0.08
Control	284.91 ±16.78	1.178 ±0.03
T-test	2.899 **	0.245 **
P-value	0.0001	0.0001

** (P≤0.01).

The study found that ferritin levels were significantly higher (P≤0.01) in people with β-thalassemia compared to controls. This finding was similar to what was found in [31], which also found that the percentage of ferritin was statistically significant in people with β-thalassemia.

Furthermore, [32] found that patients with b-thalassemia major have a high level of ferritin as a result of frequent blood transfusions and iron overload. [33] also noticed that the serum ferritin concentration dramatically rose as the number of blood transfusions increased.

Iraqi patients' significantly elevated ferritin and serum iron levels suggested an existing iron excess. His elevated ferritin level is a significant risk factor for myocardial infarction. Without chelation therapy, frequent blood transfusions in β-thalassemia cause iron overload, which results in splenomegaly and other issues. They also affect the liver's function, which raises the risk of other complications (GPT, GOT) [34].

On the other hand, C-reactive protein (CRP) is primarily used as a marker of infection and inflammation. We can measure and plot CRP readings to assess the progression of a disease or the efficacy of treatment. [35] show that IMA and CRP were significantly higher in the beta-thalassemia group compared with the control; this finding is consistent with the current research.

According to [36], there was an interesting trend toward higher levels of C-reactive protein in beta-thal/HbE post-splenectomy patients whose platelet counts were higher. Platelets, or factors that control thrombopoiesis, appear to be involved in the population's high serum C-reactive protein levels. Scientists have used C-reactive proteins and cytokines to find signs of inflammation in people with thalassemia and other illnesses, such as pneumonia, an infection that makes cystic fibrosis, diabetes, and hepatitis worse, and to see when heart problems are starting to happen.

Moreover, [37] discovered that patients with β-thalassemia intermedia had considerably higher levels of all endothelial adhesion molecules as well as CRP, and that therapy had no effect. Recent studies have consistently found higher CRP levels in thalassemia patients, particularly in splenectomized patients, compared to healthy individuals.

Table 3 summarizes the results of pro-inflammatory cytokine concentrations in β-thalassemia patients and controls in the current study. The amount of IL-1β was significantly higher (p<0.01) in people with β-thalassemia (284.19 ±4.29 pg/ml) compared to the healthy control group (142.09 ±0.70 pg/ml). Also, the IL-6 level was significantly higher (p<0.01) in the β-thalassemia patients compared to the healthy group (11.43 ±0.32 pg/ml). It was 27.92 ±0.97 pg/ml in the patients.

Table 3. Level of IL- 1β and IL-6 in β-thalassemia major patients and control

Group	Mean ± SE	
	IL-1β (pg/ml)	IL-6 (pg/ml)
Patients	284.19 ±4.29	27.92 ±0.97
Control	142.09 ±0.70	11.43 ±0.32
T-test	1.987 **	2.778 **
P-value	0.0001	0.0001

** (P≤0.01).

The results of IL-1β in the present study are supported by the results of the study done [38], where it showed an increase in the level of IL-1β compared with the control group. On the other hand, it has been demonstrated that TNF- and IL-1β play a major role in mediating endothelial cell activation. There are too many TNF-a and IL-1β in people with β-thalassemia/HbE. This could be a mononuclear phagocyte response to the sick red blood cells.

These monokines, Th1 and NK, are most likely to serve as cofactors for the synthesis of interferon gamma (IFN-g) [39]. as well as The huge rise in interleukin-1β levels in thalassemia patients in these results is similar to what was seen in the study [40]. Compared to the control group, the thalassemia patients in this study had a much higher level of IL-1β than the healthy people in the control group. Numerous significant cytokines, such as interleukin-1 beta (IL-1), are believed to be major inflammatory mediators that damage diverse organs.

Furthermore, the highly significant ($p<0.01$) increase in the level of IL-6 in the β-thalassemia patients in the current study agrees with the study [41]. The study has concentrated on the connection between IL-6 and adiponectin, positing that adiponectin serves as a potent trigger for IL-6 and TNF- secretion in primary human peripheral macrophages. Moreover, the cytokine interleukin-6 (IL-6) triggers the transformation of T helper cells into Th17 cells, a combination of macrophages and T lymphocytes that significantly contribute to the immune response. It also plays a role in both local tissue inflammation and the systemic inflammatory response [42]. Also, as found in other studies, serum levels of IL-6 were significantly higher in patients with beta-thalassemia compared to the control group [43].

4.Conclusion

Beta-Ttalassemia major is a genetic disease that affects the production of hemoglobin. It causes severe anemia in countries in the Middle East and the Mediterranean. Patients who get multiple blood transfusions end up with iron overload, which causes oxidative stress and weakens their immune systems. The pro-inflammatory cytokines, especially interleukin-6, are responsible for local inflammation and play a significant role in the progression of the disease. Also, IL-1β plays a major role in mediating endothelial cell activation and is thought to be a major inflammatory mediator that damages diverse organs.

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Conflict of Interest

There is no conflict of interest.

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Ethical Clearance

Ethics of scientific research were carried out in accordance with the international conditions followed in dealing with laboratory animals, and included animal health, husbandry and care for it, and providing appropriate conditions for it in terms of food, and appropriate methods were adopted in dealing with it when experimenting, and this is consistent with the instructions of the Iraqi Ministry of Health and Environment were enrolled in this study during their attendance at "Abin AL-Baladi Hospital in Baghdad" from September 2022 to January 2023, College of Science CSEC/0922/0104 in 2022/9/28.

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