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The association between plasma IL-6 levels and several thalassemia-related clinical features in Iraqi patients

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Abstract---The present study was set to investigate the potential association between the level of Interleukin-6 (IL-6), as a key component of the pro-inflammatory response, with different thalassemia's biological and clinical features. For this purpose, one hundred fifty blood samples were collected from 100 beta-thalassemia patients, who attended the Genetic Hematology Centre at Ibn Al- Baladi Hospital in Baghdad, Iraq, and 50 healthy subjects who were employed as a control group. IL-6 levels were estimated using an ELISA Kit, whereas other thalassemia-related clinical features (such as HbA, HbF, ferritin, blood transfusions, splenectomy status, and the history of frequent infection) were additionally assessed. The results of the present study showed a significant elevation ($P \leq 0.01$) in the levels of IL-6 in thalassemia patients as compared to healthy controls (57.7763 ± 8.94837 vs. 6.3059 ± 1.90364 pg/ml, respectively). Furthermore, IL-6 plasma levels seem to be influenced by the number of multiple scheduled blood transfusions, with the higher IL-6 mean level corresponding to the more frequent transfusions. Also, splenectomized thalassemia patients showed significantly higher IL-6 levels than those of non-splenectomized patients (61.2687 ± 9.30688 vs. 56.9571 ± 8.71926 pg/ml, respectively). Additionally, thalassemia patients exhibited a significant increase in HbF and ferritin levels in comparison to the healthy controls (57.1430 vs. 0.5020 g/dL and 2813.7000 vs. 56.8220 ng/ml, respectively), whereas the levels of HbA showed a significant

drop as compared to the control group (26.8118 vs. 96.1340 g/dL). IL-6 plasma levels correlated positively with ferritin levels ($r= 0.611$, $p\leq 0.0001$) and HbF ($r= 0.7501$, $p\leq 0.0001$). However, IL-6 appears to correlate negatively with HbA ($r= -0.885$, $P\leq 0.0001$) in β -thalassemia patients compared to healthy controls. Overall, the present investigation suggests that the levels of IL-6 in β -thalassemia cases seem to be affected by the multiple scheduled blood transfusions and other disease-associated complications, including splenectomy and elevated ferritin levels, which might influence patients' susceptibility to infections.

Keywords---Interleukin-6, beta-thalassemia, splenectomy, ferritin level, HbF, HbA, blood transfusion

Introduction

Thalassemia is an autosomal regressive disorder characterized by abnormal globin chain production. It is caused by mutations in the globin chain-encoding genes on chromosome 11. Beta-thalassemia major (β -TM) is caused by β -globin gene mutations. Reduced or absent expression of the β -globin gene results in α -globin imbalance; the insoluble α -globin precipitates as damaged molecules, leading also to kill erythroid precursors. Thalassemia is considered as one of the most common hereditary diseases worldwide, with a particularly high prevalence in tropical and subtropical areas, such as the Middle East, the Mediterranean countries, India, Southeast Asia, and North Africa. Beta-thalassemia is classified as major (BTM), intermedia (BTI), or minor (BTM), based on the clinical and genetic characteristics of the patient [1]. β -Thalassemia major from is a fatal childhood disease to a chronic disease last through life time[2]

The management of β -thalassemia involves broadly applied measures, ranging from blood transfusion, bone marrow transplantation, to splenectomy, with the primary goal is to improve hemoglobin status. Iron overload damages organs by increasing the production of reactive oxygen species (ROS), resulting in chronic oxidative stress, chronic inflammation, ferroptosis, impaired phagocytic/immune cell function, and an increased risk of immunosuppressive virus transmission; all of which result in increased morbidity and mortality in beta-thalassemia patients, particularly the major form [3]. Iron accumulation is a significant consequence of beta-thalassemia. It is caused by both blood transfusions and inefficient erythropoiesis, resulting in organ toxicity and malfunction (in the heart, liver, or endocrine glands) [4]. β -thalassemia major (TM) transfusion-dependent patients may require splenectomy. This therapeutic option is taken to minimize the blood transfusions and the resultant iron overload accordingly [5].

Interleukin-6 (IL-6), encoded by the IL-6 gene that maps to 7p15.3 in humans, is a key pro-inflammatory cytokine and acts as an anti-inflammatory myokine as well. It can be synthesized by several immune-related cells, such as macrophages and monocytes, during innate immune response as a consequence to tissue injury or infection following the recognition of pathogens through toll-like receptors (TLRs) [6].

Interleukin-6 (IL-6) has a wide impact on immune and non-immune system cells, often exhibiting hormone-like features that influence homeostatic processes. It also has context-dependent pro- and anti-inflammatory properties. Numerous cytokines have been identified in sites of persistent inflammation, including periodontitis, autoimmune disorders, thyroiditis, and rheumatoid arthritis. Although rapid production of IL-6 contributes to host defense during infection and tissue injury, it is thought that its overproduction is involved in disease pathology. Additionally, there is evidence that IL-6 is overproduced in patients with thalassemia [7]. IL-6 is a pyrogenic agent and stress signal that mediates hepatocytic synthesis of acute inflammatory phase proteins [8]. A previous study indicated that patients with thalassemia have a higher pro-resorptive level of IL-6 [9].

As a consequence of iron overload in thalassemic patients, the elevated levels of ferritin, which is an iron storage protein, are believed to cause a reduction of some of the innate immune system's proteins and this, in turn, could maximize the risk of infections. In this regard, it is thought that free and excessive iron can cause organ toxicity and damage through the formation of free radicals [10]. Thalassemia is considered as a risk factor for liver disease, including development of hepatocarcinoma, due to liver iron overload and the high prevalence of viral hepatitis [11]. The immunity of thalassemia patients is described to be impaired. This could be mostly due to the fact that the management of this disease is mainly blood transfusion-dependent, which leads to iron overload and affects vital body organs, including the spleen. The present study was set to investigate the potential association between IL-6 levels and different biological and clinical features in Iraqi β -thalassemia patients.

Method

One hundred blood samples were collected from beta-thalassemia patients who attended the Genetic Hematology Centre at Ibn Al-Baladi Hospital in Baghdad, Iraq, from November 2021 to April 2022. Patients were divided into 64 males and 36 females, including 50 cases of each of the two groups of major and intermedia, with different ages ranging between 1 and 53 years. Patients were diagnosed at the aforementioned center according to complete blood count (CBC), hemoglobin electrophoresis, ferritin tests, and disease-related clinical features examined by the center physicians and consultants. In addition, blood samples were collected from healthy individuals (without hemoglobinopathy) from the Baghdad population and used as a control group, including 19 males and 31 females of different ages ranging between 19 and 48 years. Approximately 4 ml of peripheral venous blood was taken from each patient and control subjects. Each blood sample was placed in a plain test tube for 15 minutes to separate the plasma before being centrifuged at 3,000 rpm. The collected plasma was stored at -20 °C in special Eppendorf tubes until used for assessing the level of IL-6 for all the samples by using the Human IL-6/Interleukin-6 ELISA Kit, Boster antibody, and ELISA experts, USA. The clinical and disease-related data, such as blood transfusion, splenectomy status, ferritin level, HbA level, HbF level, and frequent infection (viral) were also collected from each patient's hospital record. The study design was approved by the Research Ethical Committee of the College of Science,

University of Baghdad (No. CSEC/1021/0055). The results and data from this study were all analyzed in Excel. The Statistical Analysis System- SAS (2012) program was used to investigate the parameters of this study. t-Test was used to determine if there is a significant difference between the means of two compared groups.

Result

Elevated plasma IL-6 levels in thalassemia patients

Analysis results of plasma IL-6 levels showed a significant difference ($P < 0.01$) between β -thalassemia patients compared to the healthy controls. Levels of plasma IL-6 were able to separate thalassemia patients and healthy controls as two distinct groups (mean \pm SD 57.7763 ± 8.94837 vs. 6.3059 ± 1.90364 pg/ml, respectively, Figure 1). Further analysis of the levels of IL-6 of the studied thalassemia subtypes (major and intermedia) also showed significant differences ($P < 0.01$). The mean IL-6 level was higher in thalassemia major patients (65.1840 ± 5.73697 pg/ml) as compared to that in thalassemia intermedia patients (50.3685 ± 4.10842 pg/ml).

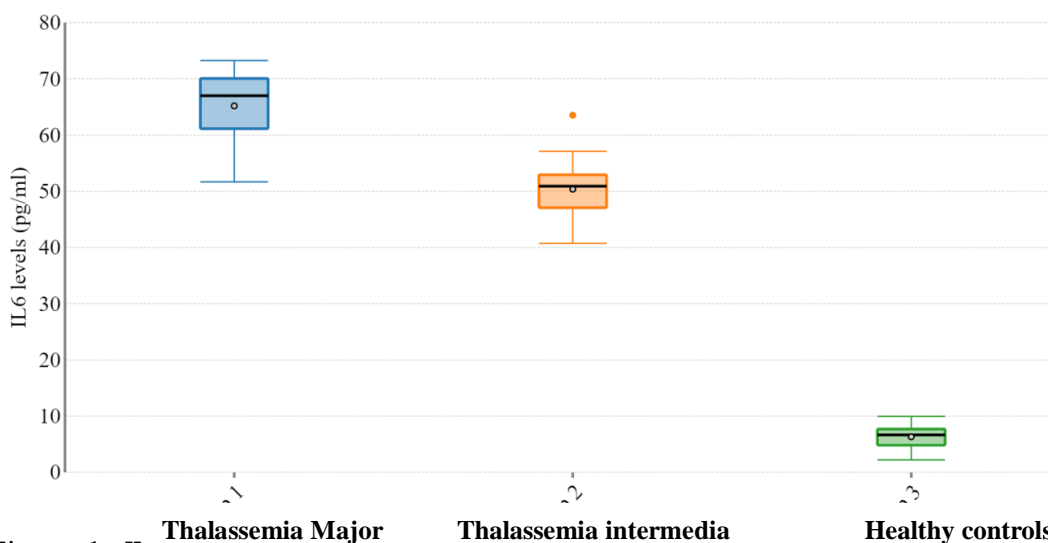


Figure 1. II patients in comparison to the healthy controls.

Mean IL-6 plasma level seems to be influenced by the schedule of blood transfusion

The levels of IL-6 seem to be proportionally affected by the duration of the scheduled blood transfusion. This was evident when the percentage level dropped by approximately 30% in thalassemia patients who required blood transfusion every eight weeks in comparison to those who needed transfusion on weekly bases (49.53 vs. 70.2 pg/ml, respectively, Figure 2). This observation suggests an association between the level of IL-6 and the duration of blood transfusion. Another support for this suggestion comes from the lower IL-6 levels in the thalassemia-intermedia patients investigated in our study who, as stated earlier,

required less frequent blood transfusions than those needed by thalassemia major patients.

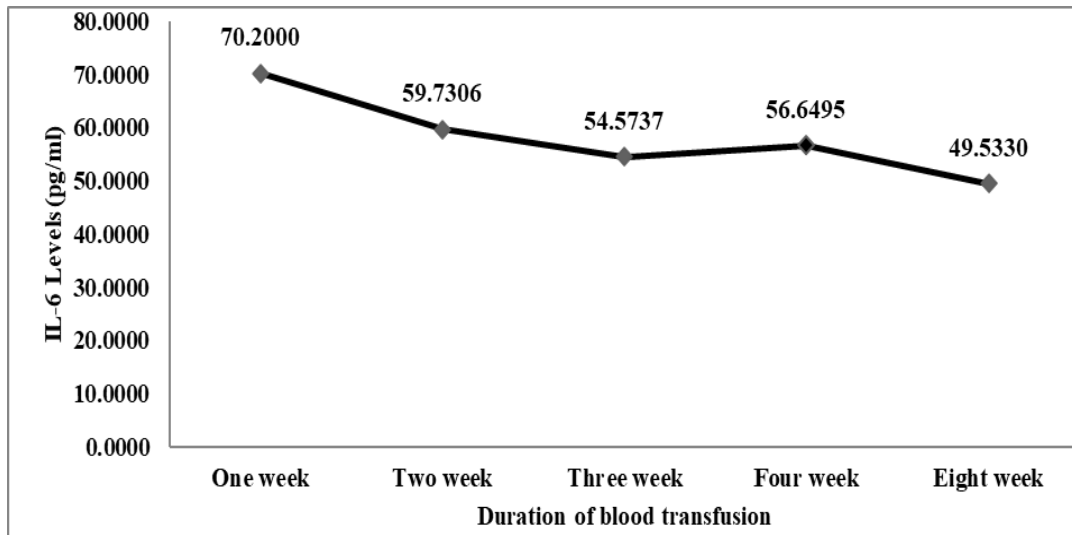


Figure 2. Plasma IL-6 levels according to the number of blood transfusions in the assessed thalassemia patients

Interleukin-6 levels did not seem to be influenced by the gender status of the assessed thalassemia patients

A comparison of the average levels of IL-6 in the investigated gender groups have provided further support to the observation that the thalassemia type has a clear impact on the IL-6 levels. This was evident since IL-6 average levels were almost comparable between both gender groups (females/males). Thalassemia females showed relatively similar IL-6 average levels to the male patients (54.9989 vs 59.3386pg/mL, respectively; $P \leq 0.01$). Similarly, no significant differences were observed in IL-6 level between genders in the healthy control group (Figure3). However, the levels of IL-6 differed significantly between genders of thalassemia major and intermedia patients. IL-6 level increased by nearly 27.7% in thalassemia major females compared with thalassemia-intermedia females ($P \leq 0.01$). In the same manner, IL-6 levels were increased by 31.4% in males with thalassemia major than those with thalassemia intermedia.

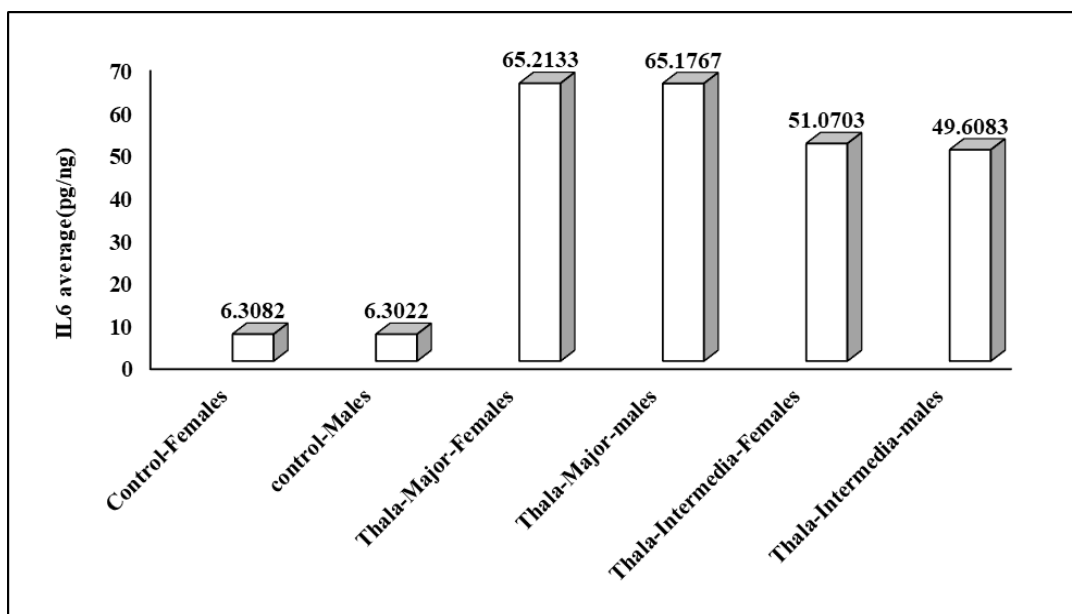


Figure 3. IL-6 levels categorized based on the gender of studied thalassemia patients in comparison to the healthy controls.

Splenectomized patients seem to have elevated IL-6 plasma level

IL-6 plasma levels seem to be increased in splenectomized in comparison to non-splenectomized thalassemia patients (61.2687 ± 9.30688 pg/ml vs. 56.9571 ± 8.71926 pg/ml, respectively, table 1). This resulted in significant differences between the two groups.

Table 1. IL-6 levels based on splenectomized and non-splenectomized thalassemia patients

Groups	IL-6(pg/ml)Means±SD
Splenectomy (n=19)	61.2687 ± 9.30688^a
Non-Splenectomy (n=81)	56.9571 ± 8.71926^b

Means with different letters in the same column differed significantly $** (P \leq 0.01)$.

Viral infection leads to elevated IL-6 plasma level in thalassemia patients

The level of IL-6 in thalassemia patients with viral infections appeared to be increased (60.1085 ± 9.81809 pg/ml) and differ significantly ($P \leq 0.01$) from those with no viral infection (57.3647 ± 8.78429 pg/ml), as shown in table 2.

Table 2. Plasma IL-6 levels in thalassemia patients based on viral infections.

IL-6 (pg/ml, Mean±SD)	
Viral infection status	
Infection (n=15)	60.1085±9.81809 ^a
Non-infection (n=85)	57.3647±8.78429 ^b

Means having different letters in the same column differed significantly ^{**}(P≤0.01).

Plasma IL-6 is positively correlated with serum ferritin in thalassemia patients

The results obtained in this study exhibited significant elevation (P≤0.01) in serum ferritin levels in thalassemia patients, in both major and intermedia groups. Serum ferritin raised by approximately 35.8% in thalassemia major in comparison to intermedia patients (3241.6000 vs.2385.8000 ng/ml, respectively, Figure 4). Our results also showed significant difference (P≤0.0001) in ferritin level in patients as compared to healthy controls. Pearson correlation coefficient was computed to assess the relationship between serum ferritin and IL-6 levels. There was a strong positive correlation between the two assessed variables (r = -0.611, P≤0.0001), implicating involvement of the increased ferritin levels in raising IL-6 plasma levels in β-thalassemia patients,

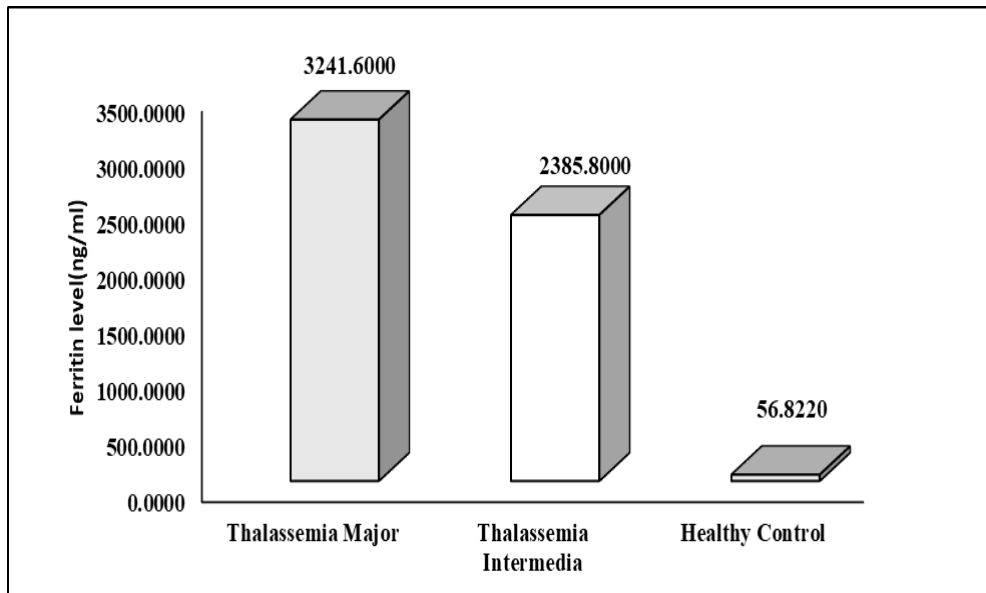


Figure 4. Serum ferritin level in thalassemia patients compared to healthy controls

Fetal Hb induction in thalassemia patients

Levels of both fetal and adult Hb in thalassemia patients were shown to be significantly different ($P \leq 0.0001$) to those in healthy controls. Fetal Hb levels were much higher in thalassemia patients than healthy controls (57.1430 vs 0.5020 g/dL, respectively, Figure 5), while those in adults were reduced by about 72% in thalassemia patients as compared to healthy controls (26.8118 vs 96.1340 g/dL, respectively). Also, IL-6 plasma levels were found to be negatively correlated with HbA ($r = -0.885$, $P \leq 0.0001$) and positively correlated with HbF ($r = 0.7501$, $p \leq 0.0001$) in thalassemia patients..

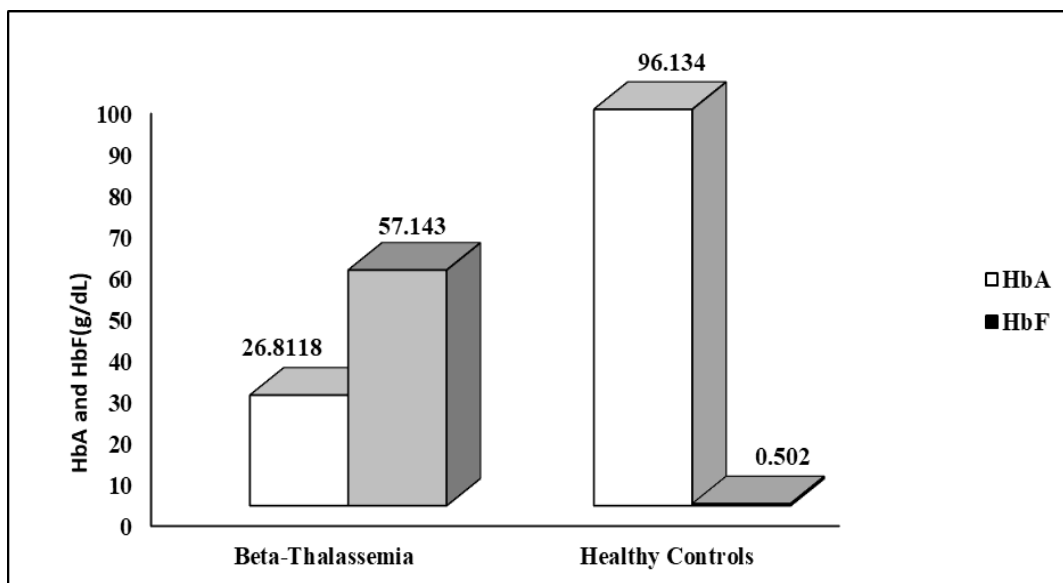


Figure 5. Fetal and adult Hb levels in thalassemia patients in comparison to healthy controls

Discussion

These increases in IL-6 levels might be a result of many immunological problems commonly reported in patients with β -thalassemia, the most significant of which include impairments of neutrophil and macrophage phagocytic activities, as well as the production of certain cytokines. Elevated IL-6 level in the serum may play a role in the pathophysiology of β -thalassemia. Increased IL-6 production is most likely due to macrophage overstimulation and may contribute to iron metabolism abnormalities [12]. The data demonstrated a substantial rise in IL-6 levels in TM patients when compared to healthy controls; this finding may be explained by the inflammation associated with frequent blood transfusions. Increased IL-6 production may be a result of persistent antigenic stimulation and iron overload associated with macrophage activation during transfusion. In this respect, our study findings seem to corroborate with that of El-Rasheidy and colleagues [13]. Similarly, a number of previous studies showed significant increases in IL-6 levels in β -thalassemia patients in comparison with healthy controls [14,

15]. However, one study reported lower levels of IL-6 in thalassemia patients than in controls [16].

While blood transfusions may be life-saving for individuals with thalassemia, it is linked with certain problems, such as iron overload and platelet and RBC alloimmunization. Typically, patients with β -thalassemia intermedia need few or no blood transfusions. They may receive transfusions on a sporadic schedule at times of sickness (e.g., sepsis) or for brief periods (e.g., during pregnancy). Patients with β -thalassemia intermedia who become transfusion-dependent follow a similar transfusion schedule to those with β -thalassemia major [17]. Early and frequent blood transfusions reduce the effects of severe anemia and increase survival in people with thalassemia. However, transfusions' beneficial effects are reduced in the long term by complications, such as chronic viral infections, hemosiderosis, and alloimmunization against RBC [18]. Multiple blood transfusions over an extended period are also known to be a significant source of immunological disorders. Indeed, recurrent blood transfusions result in chronic alloantigen stimulation, which has been linked to autoimmune hemolysis, T and B lymphocyte alterations, and monocyte and macrophage functional modifications [19]. High IL-6 plasma concentration is predictive of massive transfusion in severely injured thalassemia patients, a notion that seems to agree with the results of a recent report [20].

Although questions about gender differences in plasma IL-6 level remain unanswered at present, it is believed that it increases with age both in healthy males and females [21]. However, our study results showed that both males and females with thalassemia have higher IL-6 and the levels seemed to be influenced by the thalassemia subtype; both genders from the major subtype have higher IL-6 levels than those of intermedia and healthy controls. This finding suggests that plasma IL-6 levels are high in thalassemia regardless of the patients' gender status. Plasma IL-6 levels were also shown to increase in other diseases, such as gastric cancer, following blood reinfusions [22, 23].

Splenomegaly is one of the complications in β -thalassemia patients, and the degree of splenomegaly is related to the severity of thalassemia and the frequency of blood transfusions. As a result, splenectomy could be an option to avoid excessive spleen activity and reduce the mechanical pressure caused by an enlarged spleen. Excessive splenic activity accelerates the destruction of transfused red blood cells, resulting in an increased requirement for blood transfusion in patients. Splenectomy may increase the chance of developing thrombotic problems and acute infections after the surgical procedure. This may have occurred as a result of abnormal immunoglobulin production, changes in the activity of immune-related cells (B cells, T cells, macrophages, and neutrophils), as well as altered complement system components [24]. Splenectomized thalassemia patients are at a higher risk of infection than their non-splenectomized counterparts, as indicated by elevated levels of the inflammatory marker IL-6 [25]. On the contrary to our study finding, a previous study showed that IL-6 levels were not significantly different between splenectomized and non-splenectomized patients [26].

According to previous research, the primary predictors of infection in patients with thalassemia major are the length of thalassemia after diagnosis, the frequency of blood transfusions, splenectomy, and iron chelation. The infection episodes were more frequent in post-splenectomy than in non-splenectomy patients[27]. It is suggested that IL-6 promotes the differentiation of antibody-producing cells and that viral proteins stimulate IL-6 synthesis. In this regard, elevated IL-6 gene expression was detected in lymphoid tissues from patients with viral infection[28].

A study conducted by Saeed and colleagues showed the prevalence of viral infections (HCV, HBV, and HIV) in patients with thalassemia who required blood transfusions[29]. IL-6 is released in response to inflammation caused by viral infections; however, it has been shown that the levels of IL-6 considerably increase between 24 and 48 hours following infection[30].

Serum ferritin can be utilised as an indicator of iron status. Thalassemia patients are exposed to an increased risk of iron overload complications post-blood transfusions[31]. Long-term iron overload toxicity is often proportional to the number of transfused units[32, 33]. In this regard, our study finding seems to be in line with that of Haghpanah and colleagues who noticed that serum ferritin levels were higher in the TM group [34]. Furthermore, previous investigations stated that iron overload could be independent of blood transfusion in TI patients. The results indicated that serum levels of ferritin were extremely high in both TM and TI groups, which indicates that iron overload can cause serious health issues in thalassemia patients if left untreated. Iron overload may be a result of blood transfusion and ineffective extramedullary erythropoiesis[35, 36]. However, ferritin elevation may reflect conditions unrelated to iron metabolisms, such as inflammation, liver injury, and steatohepatitis [37]. Consistent with our finding, a previous study suggested that IL-6 may increase ferritin synthesis. Also, both ferritin and IL-6 have a significant correlation to affect serum adiponectin, CRP, Hb concentration, and platelet count [38]. Cardiac disease caused by the complications of transfusional iron overload remains the main cause of death in patients with TM over the past 25 years. Also, a significant association between serum ferritin levels and the rate of endocrinopathy was proved in a previous study [39]. It has been observed that splenectomy can affect ferritin levels. Splenectomized patients have higher ferritin levels than those in patients with an intact spleen. The intact spleen acts as a repository of extra iron and may have an excess scavenging effect on iron-free fractions, including non-transferrin iron[40].

β -thalassemia is characterized by deficit or absence of β -globin protein. In adults with thalassemia, β -globin protein is replaced by the presence of elevated HbF. This could lead to intracellular excess in free α -globin, which is believed to cause oxidative damage to the red cell membrane and apoptosis of erythroid precursors [41, 42]. In healthy individuals, about 50–80% of hemoglobin is comprised of HbF at birth, while the level continues to gradually decline after birth. By 6 months postnatally, HbF is reduced to about 8% of the total hemoglobin content. From the age of 6 months, HbA is the predominant type of hemoglobin in humans and accounts for 95–98% of the total hemoglobin molecules [43]. Similar to our study finding, a previous investigation showed a significant decrease in HbA level in thalassemia patients [44]. Low HbA levels were related to a decline in physical

function in beta-thalassemia patients, which was associated with elevated IL-6 levels [45]. IL-6 levels in the plasma of thalassemia patients appear to be correlated with fetal hemoglobin; increasing IL-6 levels lead to decreased fetal anemia [46].

Conclusion

Overall, β -thalassemia patients are at increased risk of the disease-associated complications, as manifested by the elevated plasma level of IL-6, the raise in ferritin levels, and the higher susceptibility to infections. Infection is one of the leading causes of mortality in patients with thalassemia. This imposes that a heightened index of suspicion for the increased risk of infection in patients with thalassemia, especially in those with history of splenectomy, should be maintained.

References

1. Viprakasit, V. and S. Ekwattanakit, *Clinical classification, screening and diagnosis for thalassemia*. Hematology/Oncology Clinics, 2018. **32**(2): p. 193-211.
2. Majeed, M.S., *Evaluation of some Biochemical and Endocrine Profiles in transfusion-dependent Iraqi major β -thalassemia patients*. Iraqi Journal of Science, 2017. **58**(2A): p. 639-45.
3. Keshk, W.A., et al., *Crosstalk between cytokine profile, redox, and iron status in β -Thalassemia: relation to frequency/duration of blood transfusion*. Pediatric hematology and oncology, 2019. **36**(3): p. 151-160.
4. Politou, M., et al., *The effect of transfusion on immune responses in thalassemia*. Blood Cells, Molecules, and Diseases, 2020. **83**: p. 102425.
5. Merchant, R.H., et al., *Post splenectomy outcome in β -thalassemia*. The Indian Journal of Pediatrics, 2015. **82**(12): p. 1097-1100.
6. Gubernatorova, E.O., et al., *Non-redundant functions of IL-6 produced by macrophages and dendritic cells in allergic airway inflammation*. Frontiers in immunology, 2018. **9**: p. 2718.
7. Abbas, N.A. and N.A. Zaman, *Serological Study of Human Cytomegalovirus in Thalassemia Patients and Blood Donors and its Relation to IL-6 in Kirkuk City*. Rafidain Journal of Science, 2019. **28**(1): p. 23-28.
8. Al-Zohairy, L.K. and B.N. AL-Qadhi, *Determination of Toxoplasmosis Disease Activity by Estimation of IL-6 Cytokine*. Iraqi Journal of Science, 2015. **56**(2C): p. 1600-1611.
9. Noori, N.M., M.N. Moghadam, and A. Teimouri, *TNF- α , INTERLEUKIN-6, NT-PRO BNP CORRELATION WITH ECHOCARDIOGRAPHY FINDINGS IN PATIENTS WITH THALASSEMIA*. Pakistan Heart Journal, 2019. **52**(1).
10. Moreira, A.C., G. Mesquita, and M.S. Gomes, *Ferritin: an inflammatory player keeping iron at the core of pathogen-host interactions*. Microorganisms, 2020. **8**(4): p. 589.
11. Nairz, M. and G. Weiss, *Iron in infection and immunity*. Molecular Aspects of Medicine, 2020. **75**: p. 100864.

12. Sarhat, E.R., M.S. Hamad, and S.J. Khalaf, *Leptin, Adiponectin, and Interleukin-6 Levels Among Beta Thalassaemia Patients*. Science advance, 2020. **14**(127): p. 99-105.
13. El-Rasheidy, F.H., et al., *Elevated serum adiponectin is related to elevated serum ferritin and interleukin-6 in β -thalassaemia major children*. Journal of Pediatric Endocrinology and Metabolism, 2016. **29**(8): p. 953-958.
14. Aggeli, C., et al., *Endothelial dysfunction and inflammatory process in transfusion-dependent patients with beta-thalassaemia major*. International journal of cardiology, 2005. **105**(1): p. 80-84.
15. Morabito, N., et al., *The "lively" cytokines network in β -thalassaemia major-related osteoporosis*. Bone, 2007. **40**(6): p. 1588-1594.
16. Surchi, O. and S. Ali, *Biochemical Status of Beta-Thalassaemia Major Patients in Erbil City*. Erbil Dental Journal (EDJ), 2018. **1**(1): p. 1-9.
17. Shah, F.T., et al., *Challenges of blood transfusions in β -thalassaemia*. Blood reviews, 2019. **37**: p. 100588.
18. Sadeghian, M.H., et al., *Alloimmunization among transfusion-dependent thalassaemia patients*. Asian journal of transfusion science, 2009. **3**(2): p. 95.
19. Vento, S., F. Cainelli, and F. Cesario, *Infections and thalassaemia*. The Lancet infectious diseases, 2006. **6**(4): p. 226-233.
20. Weichselbaum, N., et al., *High Interleukin-6 Plasma Concentration upon Admission Is Predictive of Massive Transfusion in Severely Injured Patients*. Journal of Clinical Medicine, 2021. **10**(11): p. 2268.
21. Legrand, D., et al., *The relationship between grip strength and muscle mass (MM), inflammatory biomarkers and physical performance in community-dwelling very old persons*. Archives of gerontology and geriatrics, 2013. **57**(3): p. 345-351.
22. Nakagawa, H., et al., *Inverse correlation between serum interleukin-6 and iron levels among Japanese adults: a cross-sectional study*. BMC hematology, 2014. **14**(1): p. 1-6.
23. Liu, J., S. Chen, and X. Ye, *The effect of red blood cell transfusion on plasma hepcidin and growth differentiation factor 15 in gastric cancer patients: a prospective study*. Annals of Translational Medicine, 2019. **7**(18).
24. Miri-Aliabad, G., A. Rezaeifar, and M. Salarzaei, *The Comparison of Immunoglobulins Status in Splenectomized and Non-Splenectomized Patients with Major Beta-Thalassaemia*. Journal of Pediatrics Review: p. 0-0.
25. Pratummo, K., et al., *Hepcidin expression from monocyte of splenectomized and non-splenectomized patients with HbE- β -thalassaemia*. Hematology, 2014. **19**(3): p. 175-180.
26. Archararit, N., et al., *Serum C-reactive protein level in postsplenectomized thalassaemic patients*. Journal of the Medical Association of Thailand= Chotmaihet Thangphaet, 2000. **83**: p. S63-9.
27. Sari, T.T., et al., *Immune response of thalassaemia major patients in Indonesia with and without splenectomy*. Acta Medica Indonesiana, 2016. **46**(3).
28. Christiansen, T., et al., *Exercise training versus diet-induced weight-loss on metabolic risk factors and inflammatory markers in obese subjects: a 12-week randomized intervention study*. American Journal of Physiology-Endocrinology and Metabolism, 2010.

29. Saeed, U., et al., *Estimation of hepatitis B virus, hepatitis C virus, and different clinical parameters in the thalassemic population of capital twin cities of Pakistan*. *Virology: research and treatment*, 2015. **6**: p. VRT. S31744.
30. Gupta, S., et al., *C-reactive Protein in Periodontitis and its Comparison with Body Mass Index and Smoking Behaviour*. *Journal of the Nepal Medical Association*, 2017. **56**(206).
31. Keikhaei, B., et al., *Relationship between serum ferritin and hemoglobin levels determined by cardiac and hepatic T2 MRI in beta-thalassemia intermedia and major patients*. *Frontiers in biology*, 2017. **12**(4): p. 311-315.
32. Cohen, A.R., E. Glimm, and J.B. Porter, *Effect of transfusional iron intake on response to chelation therapy in β -thalassemia major*. *Blood, The Journal of the American Society of Hematology*, 2008. **111**(2): p. 583-587.
33. Hershko, C., *Pathogenesis and management of iron toxicity in thalassemia*. *Annals of the New York Academy of Sciences*, 2010. **1202**(1): p. 1-9.
34. Haghpanah, S., et al., *Relationship between serum hepcidin and ferritin levels in patients with thalassemia major and intermedia in Southern Iran*. *Iranian Red Crescent Medical Journal*, 2015. **17**(7).
35. Ozment, C.P. and J.L. Turi, *Iron overload following red blood cell transfusion and its impact on disease severity*. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 2009. **1790**(7): p. 694-701.
36. Melchiori, L., S. Gardenghi, and S. Rivella, *β -thalassemia: HiJAKing ineffective erythropoiesis and iron overload*. *Advances in hematology*, 2010. **2010**.
37. Spasiano, A., et al., *Setting for "Normal" Serum Ferritin Levels in Patients with Transfusion-Dependent Thalassemia: Our Current Strategy*. *Journal of Clinical Medicine*, 2021. **10**(24): p. 5985.
38. jya YILMAZ, B.A. and E. Demiralp, *Increased plasma levels of interleukin-6 and interleukin-8 in β -thalassaemia major*. *Haematologia*, 2001. **31**(3): p. 237-244.
39. Belhoul, K.M., et al., *Serum ferritin levels and endocrinopathy in medically treated patients with β thalassemia major*. *Annals of hematology*, 2012. **91**(7): p. 1107-1114.
40. Shah, R., et al., *Serum ferritin in thalassemia intermedia*. *Indian Journal of Hematology and Blood Transfusion*, 2014. **30**(4): p. 281-285.
41. Schrier, S.L., *Pathophysiology of thalassemia*. *Current opinion in hematology*, 2002. **9**(2): p. 123-126.
42. Mabaera, R., et al., *A cell stress signaling model of fetal hemoglobin induction: what doesn't kill red blood cells may make them stronger*. *Experimental hematology*, 2008. **36**(9): p. 1057-1072.
43. Sripichai, O. and S. Fucharoen, *Fetal hemoglobin regulation in β -thalassemia: heterogeneity, modifiers and therapeutic approaches*. *Expert review of hematology*, 2016. **9**(12): p. 1129-1137.
44. Toksoy, G., et al., *Association between HBA locus copy number gains and pathogenic HBB gene variants*. *International Journal of Medical Biochemistry*, 2021. **4**(2): p. 91.

45. Wu, I.-C., et al., *Paradoxical relationship between glycated hemoglobin and longitudinal change in physical functioning in older adults: a prospective cohort study*. *The Journals of Gerontology: Series A*, 2019. **74**(6): p. 949-956.
46. Kabyemela, E.R., et al., *Fetal Cytokine Balance, Erythropoietin and Thalassemia but Not Placental Malaria Contribute to Fetal Anemia Risk in Tanzania*. *Frontiers in Immunology*, 2021. **12**: p. 1121.
47. Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, **29**(3), 550–561. <https://doi.org/10.1002/cae.22202>
48. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, **5**(3), x-xv. <https://doi.org/10.53730/ijhs.v5n3.2938>