Internal MedicineToday
Gonabad University of Medical Sciences
January 2023. Vol 29. Issue 1

Research Paper

Evaluating the Concentration of Hormonal and Cytokine Parameters in the β-thalassemia Major Patients

Shevan M. Mustafa[1], Shaban Alizadeh[1*], Ahmed H. Saleh[2], Ziba Majidi[3], Seyed Hadi Mousavi[1], Zahra Kashani Khatib[1]

1. Department of Hematology, School of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Biology, College of Science, University of Kirkuk, Kirkuk, Iraq.
3. Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran.

Use your device to scan and read the article online

Citation Mustafa Sh, Alizadeh Sh, Saleh A, Majidi Z, Mousavi SH, Kashani Khatib Z. [Evaluating the Concentration of Hormonal and Cytokine Parameters in β-thalassemia Major Patients]. Internal MedicineToday. 2023; 29(1):2-8.

:https://doi.org/10.32592/imtj.2023.29.1.2

Received: 03 Dec 2022
Accepted: 02 Mar 2023
Available Online: 28 Mar 2023

Key words:
β-thalassemia
Ferritin
Hepicidin
Interleukin
Testosterone

ABSTRACT

Aims β-Thalassemia is one of the most common groups of hereditary hemoglobinopathies. This study aimed to investigate the concentrations of hepcidin, ferritin, some interleukins (IL-6 and IL-10), and some hormones (Testosterone and follicle-stimulating hormone [FSH]) in β-thalassemia patients compared to healthy people.

Materials & Methods This study was conducted on 150 male subjects. Individuals were randomly assigned to either case group (β-thalassemia patients, n=100) or control group (healthy, n=50) based on simple randomization from Azadi Teaching Hospital and Al-Jumhuri Hospital in Iraq, from April 2022 to July 2022. Experimental work was carried out at laboratories in Kirkuk, Iraq.

Findings Our findings showed that the hepcidin and ferritin concentrations had a significant increase in the case group (P<0.05) compared to the control group. IL-6 and IL-10 concentrations demonstrated a significant rise in patients (P<0.05) compared to the control group. The concentration of sexual hormones (Testosterone and FSH) demonstrated a significant reduction in the case group (P<0.05) compared to the control group.

Conclusion β-thalassemia, in addition to hematological abnormalities, is caused by mutations in the beta gene. It is involved with other problems, such as immune, inflammatory, and endocrine imbalances. Evaluating and monitoring the biochemical, hormonal, and inflammatory factors mentioned in the present study can help prevent the iron-overload-induced consequences of major β-thalassemia.

* ***Corresponding Author:***
Shaban Alizadeh, MD.
Address: Department of Hematology, School of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran.
Tel: +98 2188982909
E-mail: alizadehs@sina.tums.ac.ir

طب داخلی روز
فصلنامه دانشگاه علوم پزشکی و خدمات درمانی گناباد

مقاله پژوهشی

بررسی غلظت پارامترهای هورمونی و سایتوکاینی در بیماران بتا تالاسمی ماژور

شوان م.مصطفی [۱]، شعبان علیزاده [۱*]، احمد ه.صالح [۲]، زیبا مجیدی [۳]، سید هادی موسوی [۱]، زهرا کاشانی خطیب [۱]

۱. گروه هماتولوژی، دانشکده پیراپزشکی، دانشگاه علوم پزشکی تهران، تهران، ایران.
۲. گروه زیست شناسی، دانشکده علوم، دانشگاه کرکوک، کرکوک، عراق.
۳. گروه علوم آزمایشگاهی، دانشکده پیراپزشکی، دانشگاه علوم پزشکی تهران، تهران، ایران.

Use your device to scan and read the article online

Citation Mustafa Sh, Alizadeh Sh, Saleh A, Majidi Z, Mousavi SH, Kashani Khatib Z. [Evaluating the Concentration of Hormonal and Cytokine Parameters in β-thalassemia Major Patients]. Internal MedicineToday. 2023; 29(1):2-8.

:https://doi.org/10.32592/imtj.2023.29.1.2

تاریخ دریافت: ۱۴۰۱/۰۹/۱۲
تاریخ پذیرش: ۱۴۰۱/۱۲/۱۱
تاریخ انتشار: ۱۴۰۲/۰۱/۰۸

چکیده

هدف: بتاتالاسمی یکی از گروه‌های شایع هموگلوبینوپاتی ارثی است. هدف از این مطالعه بررسی غلظت هپسیدین، فریتین، برخی از اینترلوکین‌ها (IL-6 و IL-10) و برخی از هورمون‌ها (تستوسترون و هورمون محرک فولیکول (FSH)) در بیماران بتاتالاسمی در مقایسه با افراد سالم است.

مواد و روش ها: این مطالعه درباره‌ی ۱۵۰ مرد بود. افراد به روش تصادفی‌سازی ساده، در یک گروه مورد (بیماران بتاتالاسمی به تعداد ۱۰۰ نفر) و یک گروه کنترل (افراد سالم به تعداد ۵۰ نفر) قرار گرفتند. نمونه‌ها از بیمارستان آموزشی آزادی و بیمارستان الجمهوری عراق، از آوریل ۲۰۲۲ تا ژوئیه‌ی ۲۰۲۲، انتخاب شدند. کارهای عملی و آزمایشگاهی در آزمایشگاه‌های کرکوک واقع در عراق انجام شد.

یافته‌ها: یافته‌های ما نشان داد که غلظت هپسیدین و فریتین در بیماران، نسبت به گروه کنترل، افزایش معنی‌داری داشت (P<۰/۰۵). غلظت IL-6 و IL-10 در بیماران، نسبت به گروه شاهد، افزایش معنی‌داری داشت (P<۰/۰۵). غلظت هورمون‌های جنسی (تستوسترون و هورمون محرک فولیکول) در بیماران، نسبت به گروه کنترل، کاهش معنی‌داری داشت (P<۰/۰۵).

نتیجه گیری: بتاتالاسمی علاوه بر ایجاد مشکلات هماتولوژیک ناشی از جهش در ژن بتا، بیماران را با مشکلات دیگری مانند بی‌تعادلی ایمنی، التهابی و غدد درون ریز درگیر می‌کند. ارزیابی و پایش عوامل بیوشیمیایی، هورمونی و التهابی ذکرشده در مطالعه‌ی حاضر می‌تواند به جلوگیری از عواقب ناشی از اضافه‌بار آهن در بتاتالاسمی کمک کند.

کلیدواژه‌ها:
اینترلوکین
بتاتالاسمی
تستوسترون
فریتین
هپسیدین

* **نویسنده مسئول:**
دکتر شعبان علیزاده
نشانی: گروه هماتولوژی، دانشکده پیراپزشکی، دانشگاه علوم پزشکی تهران، تهران، ایران.
تلفن: ۲۱۸۸۹۸۲۹۰۹ ۹۸+
پست الکترونیکی: alizadehs@tums.ac.ir

Introduction

Beta thalassemia is a monogenic disorder with defective formation in the beta chain of hemoglobin (Hb). Hb is an iron-containing protein within the red blood cells (RBCs) that transports O_2 to cells throughout the body. In an individual with β-thalassemia, a negligible degree of hemoglobin prompts a dearth of oxygen in various regions of the body. Influenced people experience a paucity of red platelets (iron deficiency), which can beget pallid dermis, frailty, lethargy, and alarming intricacies [1]. In β-thalassemia, elevated amounts of heme iron, especially of free iron, are observed. This form of iron may thus represent the trigger for the oxidative damage seen in β-thalassemia RBCs and macromolecules, including DNA, protein, and lipid membranes, and a possible consequential accelerated apoptosis may contribute to shortening the life span of thalassemia cells [2].

Hepcidin, a key component in iron hemostasis, could be a little antimicrobial peptide and encoded by the HAMP gene on 19q13, which contains 2637 bp and comprises three exons [3]. It is overwhelmingly communicated within the liver and acts by reducing the expression of ferroportin (FPN), an iron transporter on the intestinal cell surface. FPN is primarily communicated in duodenal enterocytes, liver Kupffer cells, periportal hepatocytes, and splenic macrophages. Hepcidin-FPN complex is regarded as a central negative regulator of iron egress from hepatocytes [4]. Hepcidin impacts iron homeostasis by three mechanisms; 1) inhibition of iron absorption from the duodenum, 2) blocking the release of recovered iron from macrophages and 3) controlling the movement of iron stores in hepatocytes [5]. These lead to a reduction in the amount of iron released from storage, resulting in iron being retain within the cell, thereby maintaining plasma iron levels within normal range [6]. Hepcidin levels in thalassemia patients are low, mainly due to reduced synthesis in the liver and are also affected by hematopoiesis, anemia and iron overload.

Literature review shows that one of the problems of beta-thalassemia major is iron overload, for which the ferritin molecule is the best indicator. Ferritin genes are located on chromosomes 11q and 19q. Iron is essential for the body to maintain healthy RBCs. Ferritin stores iron in the liver and immune cells. The amount of ferritin indicates the amount of iron reserves in the body. Ferritin is a protein composed of 24 subunits, among which two subunits have specifically distinct functions. The size of the light chain (L-ferritin) is 19 KDa and its gene is located on chromosome 11q; the size of the heavy chain (H-ferritin) subunit is 21 KDa and is located on chromosome 19q. The h-ferritin subunit has ferro-oxidation activity and has a site for iron binding; however, the L-ferritin subunit lacks this site. The complex of ferritin can contain 4,500 to 5,000 iron atoms [7, 8].

One of the affected aspects of β-thalassemia is the immune system, which is revealed by identifying and measuring the amount of interleukins (ILs). An imbalance in the immune system leads to inflammation and suppression of the immune system. IL-6 is a cytokine with various uses, which is a pro-inflammatory marker [9]; IL-10 is also known for its anti-inflammatory properties [10]. The evaluation of these ILs in β-thalassemia is crucial to check the immune status.

In β-thalassemia, the balance of the endocrine system is also affected, and one of the problems associated with these diseases is delayed sexual development [11]. For this purpose, we examined some hormones, such as testosterone and Follicle Stimulating Hormone (FSH).

Materials and Methods

Patients

A total of 150 individuals were reported for major β-thalassemia in Azadi Teaching Hospital and Al-Jumhuri Hospital, Iraq, from April 2022 to July 2022. Experimental work was carried out at private laboratories in Kirkuk, Iraq. The volunteers in the current study were divided based on simple randomization into two groups: healthy volunteers as the control group (n=50) and β-thalassemia patients as the case group (n=100). Groups were matched based on gender and age. The inclusion criteria were suffering from major β-thalassemia, whereas the exclusion criterion was any other type of anemia or any other related diseases.

Collection of blood samples and processing

Blood samples were collected from each patient. Scientists have differing opinions on the need for a fresh blood sample. Therefore, freshly drawn blood is not important. From each subject 5 ml venous blood sample were collected and divided into EDTA tubes (1.0 ml) and vacutainer tubes (4.0 ml). 1 ml of blood in EDTA tube was used for hematology tests (RBC, HB, HCT, and MCV) using the Sysmax device.

Vacutainer tubes were left for a short time to allow the blood to clot and then centrifuged at 4000 rpm for 10 min to obtain clear serum samples. The separated serum was placed in five tubes, sealed and stored at -20 0C until analysis. Frozen serum samples were thawed at 4-8˚C and mixed by gentle shaking at room temperature before use .

Measurements

The Hepicidin and Ferritin (Sigma-Aldrich SE120054) enzyme-linked immunosorbent assay (ELISA) kits are intended for the quantitative measurement of ferritin in human serum or plasma. Interleukins (Sigma-Aldrich

MSST0018) ELISA Kit is intended for the quantitative measurement of IL-6 and IL-10 in human serum or plasma. The Testosterone (Sigma-Aldrich SE120119) and Follicle Stimulating Hormone (FSH) ELISA kits are designed for the quantitative determination of total testosterone concentration in human serum or plasma. Our ELISA reader of choice was the LABON model from China.

Statistical Analysis

The collected data were analyzed in IBM SPSS v16.0 software. Data were presented as mean±SD (standard deviation), and values ≤ 0.05 were considered statistically significant. The normality of data distribution was checked by a Stem and Leaf Plot and Kolmogorov-Smirnov test. The results of the current study were statistically normal; therefore, we used an independent samples t-test to look for significant differences between the experimental groups by comparing their mean scores.

Results

Hepcidin and ferritin

Table 3.1 tabulates hepcidin and ferritin concentrations in β-thalassemia patients, according to which hepcidin concentration demonstrated a significant rise in patients (289.47±10.75) compared to the control group (109.28±12.64). A significant increase was observed in ferritin concentration in the case group (794.52 ± 67.13) compared to the control group (124.08 ± 22.57). In both groups, the distribution of data was normal, and we used independent samples t-tests for comparing groups.

Table 3.1. Hepcidin and ferritin in studied groups

	Control (n=50)	Case (n=100)	P-value
Hepcidin (ng/ml)	109.28±12.64	289.47±10.75	0.001
Ferritin (ng/ml)	124.08±22.57	794.52±67.13	0.008

Internal Medicine Today

The values are based on mean±SD. The data distribution was normal and analyzed based on independent samples t-test

Interleukins

Table 3.2 gives information about IL-6 and IL-10 concentrations in β-thalassemia patients; based on which IL-6 concentration demonstrated a significant boost in case group (10.73±0.46) compared to the control group (7.63±0.21). IL-10 concentration showed a significant rise in the case group (41.32 ± 1.54) compared to the control group (32.7±2.13). In both groups, the distribution of data was normal, and we used an independent samples t-test for comparing groups.

Table 3.2. Interleukin 6 and interleukin 10 concentrations in studied groups

	Control (n=50)	Case (n=100)	P-value
IL-6 (pg/ml)	7.63±0.21	10.73±0.46	0.001
IL-10 (pg/ml)	32.72±2.13	41.32±1.54	0.044

Internal Medicine Today

The values are based on mean±SD. The data distribution was normal and analyzed based on independent samples t-test

Hormones

Table 3.3 presents testosterone and FSH concentrations in β-thalassemia patients. According to this table, testosterone concentration demonstrated a significant decrease in case group (1.38±0.2) compared to the control group (5.19±0.31). It was also found that FSH concentration had a significant reduction in the case group (2.61±0.185) compared to the control group (6.73±0.29). In both groups, the distribution of data was normal, and we used independent samples t-test for comparing groups.

Table 3.3. Testosterone and FSH concentrations in studied groups

	Control (n=50)	Case (n=100)	P-value
Testosterone (ng/ml)	5.19±0.31	1.38±0.2	0.005
FSH (mIU/ml)	6.73±0.29	2.61±0.185	0.004

Internal Medicine Today

The values are based on mean±SD. The data distribution was normal and analyzed based on independent samples t-test

Discussion

Our findings showed that the concentrations of ferritin, hepcidin, IL-6, and IL-10 elevated significantly in β-thalassemia patients (P<0.05) compared to the control group. However, sexual hormones (testosterone and FSH) concentration demonstrated a significant reduction in β-thalassemia patients (P<0.05) compared to the control group.

Ferritin is a molecule that indicates the amount of iron stores, and its decrease is presented as iron deficiency anemia, hypothyroidism, and vitamin C deficiency, whereas its increase leads to such conditions as increased metabolism, tissue damage, and iron overload [12]. A rise in ferritin is observed in β-thalassemia patients due to repeated blood transfusions. To prevent iron overload complications, it is necessary to evaluate its amount and become aware of its increase. The findings of the present study about ferritin levels are in line with those reported by Pootrakul et al. [13], who found that serum ferritin levels in β-thalassemia patients were higher than the normal level. The same result was found by Mishra and Tiwari [14], who found that the serum ferritin levels in multi-transfused thalassemia

major patients in Bhopal Hospital, India, were higher than that in the control group. Easa [15] also studied patients with β-thalassemia, including major and intermediate types, who attended to Thalassemia Center in Karbala Teaching Hospital. The results of this study, which was conducted on splenectomy patients, also revealed high ferritin levels for splenectomies β-thalassemia major patients. Examining ferritin in β-thalassemia major patients is highly important due to the fact the ferritin level can be related to various factors, such as the level of iron stores, atrial electrocardiographic markers, and endocrinopathy [16].

Hepcidin is a protein with 25 amino acids rich in cysteine, is synthesized in hepatocytes, and its concentration increases under the influence of inflammation and inflammatory cytokines [17, 18]. When the amount of iron is high, hepcidin inhibits the action of ferroportin and prevents intestinal absorption of iron. In β-thalassemia major patients with occasional blood transfusion, an increase in serum hepcidin levels is expected, while, in β-thalassemia patients without blood transfusion, no significant increase in hepcidin levels is observed. The amount of hepcidin in patients with β-thalassemia major can change based on the time of blood transfusion [19]. In this study, the serum level of hepcidin was high in the β-thalassemia major group compared to normal people, which indicated the high iron overload in the studied patients. Moreover, there was no significant association between the serum levels of ferritin and hepcidin. A similar result was obtained by Haghpanah et al., ho reported that the serum level of ferritin was higher in the β-thalassemia major group [20].

Iron overload and alloimmunization should be taken into account as potential consequences when treating individuals with β-thalassemia via blood transfusion [21]. As a result, it is important to investigate the ILs involved in the immune system. The results of this study demonstrated that there was a significant increase in the serum IL-6 level in the β-thalassemia patients compared to normal subjects. These increments in the serum level of IL-6 that can be found in patients with β-thalassemia might be attributed to several immunological defects, among which the impairment of neutrophil and macrophage phagocytic as well as killing functions and the production of some cytokines are the most important. IL-6 has been found to be a crucial component of the pro-inflammatory response, and its elevated serum level may be associated with the pathophysiology of β-thalassemia [22]. Increased production of IL-6 is likely due to the overstimulation of macrophages and may contribute to abnormalities in iron metabolism [23]. The results of our study were in line with those of other studies [22, 24]. In a study, the effect of iron chelating drugs on IL-6 was evaluated and it was observed that the use of these drugs increased the level of IL-6 in β-thalassemia patients [25].

IL-10 is an anti-inflammatory cytokine and is produced by a large number of immune cells, such as $CD8^+$ and $CD4^+$ T cells, NK cells, NK T cells, B cells, dendritic cells, eosinophils, mast cells, and monocytes cells [26]. Here, we evaluated the serum level of IL-10 in β-thalassemia patients. Our results suggested that the serum level of IL-10 was significantly increased compared to the control group. This may be attributed to various factors, such as splenectomy, iron overload, and repeated exposure to foreign antigens at the time of blood transfusion, or multiple blood transfusions may expose them to dangerous infections, such as HIV, HBV, and HCV [27]. According to Balouchi et al. [28], IL-10 levels were significantly higher in β-thalassemia major patients than in healthy subjects. They also highlighted that immunological abnormalities were characterized in β-thalassemia major patients, many of which were linked to cytokines, and this imbalanced immune condition involved inflammation and immunosuppression in patients. Similarly, Voskaridou et al. [29] observed that IL-10 levels increased in patients with β-thalassemia major and IL-10 had a role in the pathogenesis of these disorders. In these patients, blood transfusion disrupts immunomodulation and causes cytokine changes, especially IL-10.

The results of this study showed a significant decrease in the serum Testosterone and FSH hormone levels. Due to the accumulation of iron in gonadotropic cells, people with beta thalassemia major exprience problems with sexual development such as: puberty failure, amenorrhea, slow or no sexual development, sexual dysfunction and infertility due to hypogonadism [30, 31]. The significant increase in ferritin levels in this study is consistent with the function of many other gonads and pituitary glands. Studies have confirmed primary hypogonadism due to iron deposition in the gonads. Secondary hypogonadism results from iron deposition on gonadotrophic cells of the pituitary gland, as demonstrated by the limited response of luteinizing hormone (LH) and FSH to gonadotropin-releasing hormone (GnRH) stimulation or a combination of both primary and secondary hypogonadisms. The abnormal gonadotrophin response pulsatilely administered GnRH clearly indicates failure of gonadotroph cells, which apper to be extremely vulnerable to iron damage [32]. According to research by T. Hamed and et al, the prevalence of hypogonadism in patients with beta thalassemia major patients is 75% [33]. consistent with our results, AL-Mahdawi and et al, found decreased FSH and Testosterone levels in patients with beta thalassemia [34]. A similar study was performed by Soliman et al [35], who reported that thalassaemic patients had a total absent of pubertal changes in 73% of boys and 42% of girls with thalassemia of age from 13 to

21 years. De Sanctis et al [36] reported that a significant proportion (30%) of patients with beta thalassemia major had hypogonadotropic hypogonadism.

Conclusion

β-thalassemia, in addition to hematological abnormalities, is caused by mutations in the beta gene. It is involved with other problems, such as immune, inflammatory, and endocrine imbalances. Preventing iron-overload-induced consequences of major β-thalassemia can be achieved by evaluating and monitoring the biochemical, hormonal, and inflammatory factors mentioned in the present study. An increase in ferritin and hepcidin indicates complications of iron balance in these patients. An increase in IL-6 and IL-10 also demonstrates the effect of iron overload on the inflammatory and immune system. Another axis of disorder caused by the accumulation of iron is the imbalance of endocrine hormones, which is indicated by a decrease in sex hormones, such as testosterone and FSH. Measuring the levels of the mentioned factors is crucial in informing people to start iron chelating treatment or following the treatment and its dosage.

Research limitations

The comparison of ferritin and hepcidin levels between the two groups of patients with thalassemia major and the control group was the main strength of this study since it can demonstrate how the type of thalassemia affects iron status markers. The lack of numerous measures of hepcidin at different times was one of the weaknesses of the study, along with the small number of samples in the control group. It is better to study this project in more groups, for example, women and men.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles of the Helsinki Declaration were observed, and the research was approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (IR.TUMS.SPH.REC.1401.127).

Funding

This project was approved by the Tehran University of Medical Sciences. Sampling was referred to Azadi Teaching Hospital and Al-Jumhuri Hospital in Iraq. Experiments were performed at the Kirkuk, Iraq, in 2022.

Authors' contributions

All authors have contributed to the design, execution, and writing of all sections of the current study.

Conflicts of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to thank all those who participated in this study.

References

[1] Ali S, Mumtaz S, Shakir HA, Khan M, Tahir HM, Mumtaz S, et al. Current status of beta-thalassemia and its treatment strategies. Molecular Genetics & Genomic Medicine. 2021; 9(12): e1788. [DOI: 10.1002/mgg3.1788] [PMID] [PMCID]

[2] Srinoun K, Svasti S, Chumworathayee W, Vadolas J, Vattanaviboon P, Fucharoen S, et al. Imbalanced globin chain synthesis determines erythroid cell pathology in thalassemic mice. haematologica. 2009; 94(9): 1211-9. [DOI: 10.3324/haematol.2009.005827] [PMID] [PMCID]

[3] Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science (New York, NY). 2004; 306(5704): 2090-3. [DOI: 10.1126/science.1104742] [PMID]

[4] Nemeth E, Ganz T. Hepcidin and iron-loading anemias. haematologica. 2006; 91(6): 727-32. [PMID]

[5] Reichert CO, da Cunha J, Levy D, Maselli LMF, Bydlowski SP, Spada C. Hepcidin: homeostasis and diseases related to iron metabolism. Acta haematologica. 2017; 137(4): 220-36. [DOI: 10.1159/000471838] [PMID]

[6] Sangkhae V, Nemeth E. Regulation of the iron homeostatic hormone hepcidin. Advances in nutrition. 2017; 8(1): 126-36. [DOI: 10.3945/an.116.013961] [PMID] [PMCID]

[7] Honarmand Ebrahimi K, Hagedoorn P-L, Hagen WR. Unity in the biochemistry of the iron-storage proteins ferritin and bacterioferritin. Chemical reviews. 2015; 115(1): 295-326. [DOI: 10.1021/cr5004908] [PMID]

[8] Plays M, Müller S, Rodriguez R. Chemistry and biology of ferritin. Metallomics. 2021; 13(5): mfab021. [DOI: 10.1093/mtomcs/mfab021] [PMID] [PMCID]

[9] Cronstein BN. Interleukin-6. Bulletin of the NYU hospital for joint diseases. 2007; 65(1): S11-5. [PMID]

[10] Sabat R, Grütz G, Warszawska K, Kirsch S, Witte E, Wolk K, et al. Biology of interleukin-10. Cytokine & growth factor reviews. 2010; 21(5): 331-44. [DOI: 10.1016/j.cytogfr.2010.09.002] [PMID]

[11] El-Hazmi MAF, Warsy A, Al-Fawaz I. Iron-endocrine pattern in patients with β-thalassaemia. Journal of tropical pediatrics. 1994; 40(4): 219-24. [DOI: 10.1093/tropej/40.4.219] [PMID]

[12] Fargion S, Taddei M, Gabutti V, Piga A, Di Palma A, Capra L, et al. Early iron overload in beta-thalassaemia major: when to start chelation therapy? Archives of Disease in Childhood. 1982; 57(12): 929-33. [DOI: 10.1136/adc.57.12.929] [PMID] [PMCID]

[13] Pootrakul P, Vongsmasa V, La-Ongpanich P, Wasi P. Serum ferritin levels in thalassemias and the effect of splenectomy. Acta haematologica. 1981; 66(4): 244-50. [DOI: 10.1159/000207129] [PMID]

[14] Mishra AK, Tiwari A. Iron overload in beta thalassaemia major and intermedia patients. Maedica. 2013; 8(4): 328-32. [PMID] [PMCID]

[15] Easa ZO. Complications of high serum ferritin level after splenectomy in β thalassemic patients. Kufa Medical Journal. 2009; 12(1): 138-42. [Link]

[16] Ghandi Y, Eghbali A, Rafie F. Correlation between mean serum ferritin levels and atrial electrocardiographic markers in beta-thalassemia major. Iranian Journal of Blood and Cancer. 2018; 10(3): 76-81. [Link]

[17] Agarwal AK, Yee J. Hepcidin. Advances in chronic kidney disease.

2019; 26(4): 298-305. [DOI: 10.1053/j.ackd.2019.04.005] [PMID]
[18] Ueda N, Takasawa K. Impact of inflammation on ferritin, hepcidin and the management of iron deficiency anemia in chronic kidney disease. Nutrients. 2018; 10(9): 1173. [DOI: 10.3390/nu10091173] [PMID] [PMCID]
[19] Nemeth E. Hepcidin in β-thalassemia. Annals of the New York Academy of Sciences. 2010; 1202(1): 31-5. [DOI: 10.1111/j.1749-6632.2010.05585.x] [PMID] [PMCID]
[20] Eshagh Hossaini SK. Association between serum levels of hepcidin and ferritin in patients with thalassemia major and intermedia, the role of iron chelator. Journal of Hematopathology. 2019; 12(3): 143-7. [DOI: 10.1007/s12288-022-01533-1] [PMID] [PMCID]
[21] Politou M, Komninaka V, Valsami S, Kapsimali V, Pouliakis A, Koutsouri T, et al. The effect of transfusion on immune responses in thalassemia. Blood Cells, Molecules, and Diseases. 2020; 83: 102425. [DOI: 10.1016/j.bcmd.2020.102425] [PMID]
[22] Sarhat ER, Hamad MS, Khalaf SJ. Leptin, Adiponectin, and interleukin-6 levels among beta thalassaemia patients. Science advance. 2020; 14(127): 99-105. [Link]
[23] Oztürk O, Yaylim I, Aydin M, Yilmaz H, Agaçhan B, Demiralp E, et al. Increased plasma levels of interleukin-6 and interleukin-8 in beta-thalassaemia major. Haematologia (Budap). 2001; 31(3): 237-44. [DOI: 10.1163/15685590152763782] [PMID]
[24] Aggeli C, Antoniades C, Cosma C, Chrysohoou C, Tousoulis D, Ladis V, et al. Endothelial dysfunction and inflammatory process in transfusion-dependent patients with beta-thalassemia major. International journal of cardiology. 2005; 105(1): 80-4. [DOI: 10.1016/j.ijcard.2004.12.025] [PMID]
[25] Hassan AM, Rahman SJA. Evaluation of liver enzymes, interleukin-6 and tumer necrosis factor-alpha in children suffering from thalassemia and treated with Deferoxamine and Deferasirox drug in Kirkuk city. HIV Nursing. 2022; 22(2): 1461-5. [Link]
[26] Saraiva M, Vieira P, O'garra A. Biology and therapeutic potential of interleukin-10. Journal of Experimental Medicine. 2020; 217(1). [DOI: 10.1084/jem.20190418] [PMID] [PMCID]
[27] Hashim HH, Jamal QW, Alrawi FA. Evaluation of Interleukins 12 and 13 levels in beta Thalassemia major patients and their relations to viral Hepatitis C. Iraqi Journal Of Medical Sciences. 2015; 13(1): 40-4. [Link]
[28] Balouchi S, Gharagozloo M, Esmaeil N, Mirmoghtadaei M, Moayedi B. Serum levels of TGFβ, IL-10, IL-17, and IL-23 cytokines in β-thalassemia major patients: the impact of silymarin therapy. Immunopharmacology and immunotoxicology. 2014; 36(4): 271-4. [DOI: 10.3109/08923973.2014.926916] [PMID]
[29] Voskaridou E, Christoulas D, Papatheodorou A, Plata E, Xirakia C, Tsaftaridis P, et al. Angiogenic molecules and inflammatory cytokines in patients with thalassemia major and double heterozygous HbS/Beta-thalassemia; the impact of Deferasirox. Blood. 2009; 114(22): 2018. [DOI:10.1182/blood.V114.22.2018.2018]
[30] Costin G, Kogut MD, Hyman CB, Ortega JA. Endocrine abnormalities in thalassemia major. American Journal of Diseases of Children. 1979; 133(5): 497-502. [DOI: 10.1001/archpedi.1979.02130050041009] [PMID]
[31] Mahmud AA, Al-Gharawi AM, Mustafa FM. Evaluation of delayed puberty of patients with beta thalassemia major in–diyala governorate. Diyala Journal of Medicine. 2018; 15(2): 76-9. [Link]
[32] Vidal A, Dhakal C. Association of beta-thalassaemia and hypogonadotropic hypogonadism. Case Reports in Obstetrics and Gynecology. 2022; 2022 :4655249. [DOI: 10.1155/2022/4655249] [PMID] [PMCID]
[33] Hamed AT, Zughbur MR, Shaheen EA. Prevalence of hypogonadism in β-thalassemia major patients from Gaza strip. Jordan Journal of Pharmaceutical Sciences. 2021; 14(3). [Link]
[34] AL-Mahdawi FKI, Elia ZN, Kurji HA. Evaluation inhibin B, FSH, and LH in male with thalassemia. Indian Journal of Forensic Medicine & Toxicology. 2020; 14(2): 2437-40. [DOI: 10.37506/ijfmt.v14i2.3401]
[35] Soliman AT, Elzalabany M, Amer M, Ansari B. Growth and pubertal development in transfusion-dependent children and adolescents with thalassaemia major and sickle cell disease: a comparative study. Journal of tropical pediatrics. 1999; 45(1): 23-30. [DOI: 10.1093/tropej/45.1.23] [PMID]
[36] De Sanctis V, Pintor C, Gamberini M, Ughi M, Pinamonti A, Aliquo M, et al. Multicentre study on prevalence of endocrine complications in thalassaemia major. Clinical Endocrinology. 1995; 42(6): 581-6. [DOI: 10.1111/j.1365-2265.1995.tb02683.x] [PMID]