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Evaluation of some hormones in patients with major β-Thalassemia in the Nineveh Governorate - Iraq

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ABSTRACT. Thalassemia is one of the most important genetic haemolytic diseases that cause the breakdown of red blood cells (RBCs) in patients with β-Thalassemia major. The body does not produce enough haemoglobin, which is an important part of RBCs. When there is not enough haemoglobin, RBCs do not function properly in the body, so the condition continues for short periods of time. The current studies aimed to determine the extent of the impact of β-Thalassemia major on some hormonal variables in the serum of 80 patients (40 males and 40 females) aged between (1-15) years, in addition to 20 healthy children of the same age range and of both sexes, who were considered as a control group. The results of this study showed a significant increase in the concentration of erythropoietin (EPO) by 187% in the serum of patients with β-Thalassemia major compared to healthy of both sexes, with an increase of 188 in males and 183% in females. The highest significant increase was in the age group of (11-15) years in males and females compared to healthy control. The results also showed a significant decrease in the concentration of hepcidin and growth hormones in the serum of patients with a decrease of 55 and 56% respectively compared to healthy individuals of both sexes, with a highest significant decrease of 56 and 59% in males, and 55 and 52% in females respectively. The highest significant decrease was in the age group of (11-15) years for both hormones in males and females compared to healthy control based on age groups and sex.

Keywords: β-Thalassemia major; erythropoietin hormone; growth hormone; hepcidin hormone; Red Blood Cells (RBCs).

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Introduction

β-Thalassemia major is one type of Thalassemia that results from a decrease or absence of the biosynthesis of Beta-globin chains (β-Globin) (Polat, 2021; Hesham, El-Safy, El-Taweel, & Omar, 2022). It is one of the most widespread diseases globally, and it is prevalent in Arab countries and Mediterranean countries such as Italy, Greece, Turkey, Iran, and North African countries (De Sanctis et al., 2017).

In the case of β-Thalassemia major, there is an increase in the formation of red blood cells (RBCs), while the ineffective formation of RBCs is a fundamental feature for patients with β-Thalassemia major. Patients suffer from a severe decrease in haemoglobin concentration and severe anemia, accompanied by splenomegaly and bone deformities due to the acute deficiency in hemoglobin production (Rivella, 2015; Brancaleoni, Di Pierro, Motta, & Cappellini, 2016; Hamed, Al-Taii, & Jankeer, 2021).

Erythropoietin hormone is a glycoprotein produced by the kidneys, mainly from interstitial cells of the fibroblast associated with peritubular capillaries in the nephron (Lundby & Olsen, 2011). It regulates and stimulates the production of RBCs in the bone marrow. Several studies have indicated that erythropoietin hormone plays a role in protecting against inflammation, acts as an antioxidant to protect against oxidative stress (Gholamzadeh, Eskandari, Bigdeli, & Mostafavi, 2018), and has a significant role in controlling the differentiation process and maintaining the stability of RBC numbers and the survival of blood cell progenitors (Lin, Luo, Jin, Shi, & Gong, 2015). In addition, its basic and important role in erythropoiesis in the bone marrow in mammals and responds to hypoxia within tissues by activating the hypoxia-inducible factor (HIF). Erythropoietin levels are low in the absence of anaemia, but exposure to stress due to oxygen deficiency significantly increases the production of erythropoietin (Haase, 2013).

Hepcidin hormone is a peptide, it is mainly produced by hepatic cells (Clark et al., 2011). It is the main regulator of iron availability in the body, helping absorb iron from the duodenum, promoting phagocytic cells to recycle old RBCs, storing excess iron in hepatic cells, and ensuring a feedback loop between iron and hepcidin to maintain stable iron concentrations in the plasma (Ganz, 2006). It also plays a crucial role in iron

metabolism, with its importance in iron metabolism lying in its ability to control iron release from cells (Ambachew & Biadgo, 2017). In cases of anaemia, Thalassemia, and hypoxia, hepcidin increases the production of RBCs. Anaemia increases the decrease in the level of the hormone, and a decrease in hepcidin level raises the level of iron absorbed by the digestive tracts and stored in hepatic cells, indicating that hepcidin hormone contributes directly to iron metabolism (Courselaud et al., 2002).

Growth hormone is a protein peptide hormone secreted of the anterior pituitary gland, affecting most tissues in the body, it also affects the pathway of catabolism lipid, protein synthesis, anaemia, and decreased glucose synthesis (Caputo et al., 2021). It also has a direct effect on bone growth (Krohn et al., 2003). A decrease in growth hormone level is a characteristic feature of β-Thalassemia, as there is high toxicity to the pituitary gland due to the high level of iron (De Sanctis, 2002). Several studies have indicated delayed growth in patients with β-Thalassemia major, attributed to a decrease in insulin and insulin-like growth factor-1 (IGF-1) level (Wu, Tsai, & Peng, 2003; Soliman et al., 2011).

Materials and methods

Chemical material used

This study used ready-made kits from different international companies to estimate some hormonal variables, including erythropoietin, hepcidin, and growth hormones. These kits were provided by SunLong Biotech LTD, a Chinese company, and DRG International Inc., an American company.

Location and duration of study

This study was conducted on patients with β-Thalassemia major in the Thalassemia Center at Al Hadbaa Blood and BMT Hospital in Nineveh Governoraten Nineveh Governorate - Iraq, from January (2022) to June (2022). The study included 80 patients (40 males and 40 females) aged between (1-15) years. The patients were diagnosed by specialist doctors depending on the laboratory tests of blood film, electrophoresis, iron levels and their family history of the disease was also considered. The patients were divided into three groups based on age categories using a specific questionnaire for this disease. The groups were classified into 3 groups for both genders, males and females:

- The first group included 25 patients with β-Thalassemia major in the age group of (1-5) years.
- The second group included 30 patients with β-Thalassemia major in the second age group of (6-10) years.
- The third group included 25 patients with β-Thalassemia major in the age group of (11-15) years.

The study also included 20 healthy children of the same age range and gender as the patients, who did not have this disease after laboratory tests were conducted by a specialist doctor, and they did not have a family history of anaemia or genetic blood disorders, who were considered as a control group.

Collection and preservation of blood sample

Blood samples were collected between (8-11) am, and (4-5) mL of venous blood was drawn before giving the blood to the patient using a 5 mL syringe. The blood was then placed in jell plastic tubes, free from any anti-coagulant material, and left at room temperature for 30 min. to clot. The blood was then separated using a centrifuge for 15 min. at a speed of 3000 revolutions per min. to obtain serum. The serum was then drawn using a micropipette and placed in plastic Eppendorf tubes, and stored in the freezer at a temperature of (-20)°C until hormonal tests were conducted.

Statistical analysis

The statistical analysis of the results was performed using the Complete Randomized Design (C.R.D) and differences between the patients and control group were determined using the t-test for the studied variables at a probability level of ($p \leqslant 0.01$). Meanwhile, the differences between the patient groups and the control based on gender and age categories were determined using Duncan's Multiple Range Test at a probability level of ($p \leqslant 0.05$), and significant differences were determined using the SAS statistical program to find the mean and standard error (SE) (Hinton, 2004).

Results

The results in Table 1 showed a significant increase at a probability level of ($p \leqslant 0.01$) in the concentration of erythropoietin (EPO) hormone as the percentage of increase was 187% in the blood serum of patients with β-Thalassemia major compared to healthy in both sexes. The results in Table 2 also showed a significant increase at a probability level of ($p \leqslant 0.05$) in the concentration of EPO hormone, as its increase in males and females reached 188% and 183% respectively, compared to healthy control and by gender. While the results in Table 3 showed a significant increase at a probability level of ($p \leqslant 0.05$) in the concentration of EPO for all studied age groups, including (1-5), (6-10) and (11-15) years, for both sexes. The highest percentage of increase in females in the age group (11-15) years reached 279%, while the highest percentage of increase in males in the same age group reached 207% compared to healthy control, according to age groups and gender.

Table 1. Concentration of some hormones in the serum of patients with β-Thalassemia major compared to healthy control of both sexes.

Studied Groups	Control			Patients		
Hormones	Concentration Mean ± SE	% Concentration	% Change	Concentration Mean ± SE	% Concentration	% Change
Erythropoietin Hormone EPO (pg mL^{-1})	68.7 ± 11.4	100	-	196.9 ± 38.9**	287	+187
Hepcidin Hormone (ng mL^{-1})	73.5 ± 6.2	100	-	33.1 ± 13.3**	45	-55
Growth hormone GH (IU mL^{-1})	7.9 ± 1.3	100	-	3.5 ± 1.4**	44	-56

The numbers followed by the sign (**) indicate significant differences at ($p \leqslant 0.01$) according to (t-Test). The sign (-) means a decrease. The sign (+) means an increase.

Table 2. Concentration of some hormones in the serum of patients with β-Thalassemia major compared to healthy control according to gender.

Studied Groups		Control			Patients		
Hormones	Gender	Concentration Mean ± SE	% Concentration	% Change	Concentration Mean ± SE	% Concentration	% Change
Erythropoietin Hormone EPO (pg mL^{-1})	Male	73.9 ± 9.0a	100	-	207.6 ± 39.6a	288	+188
	Female	63.7 ± 14.1a	100	-	180.1 ± 32.2a	283	+183
Hepcidin Hormone (ng mL^{-1})	Male	71.8 ± 5.4a	100	-	31.3 ± 12.1b	44	-56
	Female	76.9 ± 6.9a	100	-	34.9 ± 14.8b	45	-55
Growth hormone GH (IU mL^{-1})	Male	8.0 ± 1.4a	100	-	3.3 ± 1.3b	41	-59
	Female	7.9 ± 1.1a	100	-	3.8 ± 1.4a	48	-52

The numbers followed by different letters horizontally indicate a significant difference at ($p \leqslant 0.05$) according to Duncan's Test. The sign (-) means a decrease. The sign (+) means an increase.

Table 3. Concentration of some hormones in the serum of patients with β-Thalassemia major compared to healthy control according to age groups and gender.

Studied Groups	Gender		Age Categories								
			(1-5) Year			(6-10) Year			(11-15) Year		
Hormones			Concentration Mean ± SE	% Concentration	% Change	Concentration Mean ± SE	% Concentration	% Change	Concentration Mean ± SE	% Concentration	% Change
Erythropoietin Hormone EPO (pg mL^{-1})	Male	Control	70.1 ± 11.5b	100	-	72.5 ± 1.8b	100	-	73.3 ± 11.8b	100	-
		Patients	185.0 ± 35.3a	264	+164	198.9 ± 7.2a	274	+174	225.2 ± 42.5a	307	+207
	Female	Control	69.5 ± 18.2b	100	-	62.7 ± 17.3b	100	-	54.1 ± 0.07b	100	-
		Patients	165.6 ± 22.5a	238	+138	177.8 ± 34.1a	284	+184	204.8 ± 33.7a	379	+279
Hepcidin Hormone (ng mL^{-1})	Male	Control	67.8 ± 6.4a	100	-	73.7 ± 5.5a	100	-	73.3 ± 4.1a	100	-
		Patients	31.5 ± 7.7b	46	-54	34.1 ± 11.6b	46	-54	30.0 ± 14.4b	41	-59
	Female	Control	81.7 ± 2.1a	100	-	73.0 ± 10.6a	100	-	75.4 ± 0.09a	100	-
		Patients	39.9 ± 17.8b	49	-51	35.5 ± 17.5b	49	-51	28.1 ± 8.3b	37	-63
Growth hormone GH (IU mL^{-1})	Male	Control	8.1 ± 1.7a	100	-	8.5 ± 1.1a	100	-	7.5 ± 1.7a	100	-
		Patients	4.8 ± 1.0b	59	-41	3.4 ± 0.5bc	40	-60	2.3 ± 0.8c	31	-69
	Female	Control	7.1 ± 1.7a	100	-	8.5 ± 0.2a	100	-	8.3 ± 0.07a	100	-
		Patients	5.0 ± 0.8a	70	-30	3.9 ± 1.5bc	46	-54	2.5 ± 0.4c	30	-70

The numbers followed by different letters horizontally indicate a significant difference at ($p \leqslant 0.05$) according to Duncan's Test. The sign (-) means a decrease. The sign (+) means an increase.

The results of the current study are consistent with the findings of previous studies (Chaisiripoomkere, Jootar, Chanjarunee, & Ungkanont, 1999; Amer, Dana, & Fibach, 2010; Nguyen & Nguyen, 2017), which indicates a significant increase in the concentration of erythropoietin hormone in the blood serum of patients with β-Thalassemia major compared to healthy control. This increase is attributed to several factors,

including the severity of anaemia, high ferritin level, splenectomy, as well as oxygen deficiency or hypoxia (Çetin, Ünal, Gümrük, Gürgey, & Altay, 2009). The secretion of erythropoietin hormone increases if the level of arterial oxygen decreases and the total number of red blood cells decreases. One of the most common causes that lead to a lack of oxygen is anaemia, increased binding of oxygen to haemoglobin, in addition to poor blood flow in the tissues (Duchnowska & Szczylik, 2003). A significant increase in the level of erythropoietin affects the activity of enzymes involved in heme biosynthesis and haemoglobin production (Huang et al., 2019).

Erythropoietin is secreted primarily from the kidney by interstitial fibroblast cells in the renal cortex, and a small amount is also secreted from the blood vessels surrounding the cells and tissues of the liver and brain. It affects the bone marrow, and an increase in the concentration of erythropoietin hormone in the blood serum of patients with β-Thalassemia major, it leads to the formation of abnormal red blood cells that contain excess alpha-globin chains. In addition, an increase in the hormone leads to damage to the membrane of red blood cell and shortening their lifespan (Breymann et al., 1999; Amer et al., 2010; Souma, Suzuki, & Yamamoto, 2015). Several studies have shown that erythropoietin hormone has an antioxidant effect in normal conditions in two ways. Firstly, directly by stimulating the increased activity of enzymatic antioxidants such as glutathione peroxidase, catalase, and superoxide dismutase, thus, it enhances the protection system against oxidants (Katavetin et al., 2007). Second, indirectly by inhibiting iron-dependent oxidation damage, via iron consumption in the process of formation young red blood cells (Bahadorimonfared, Alirezaei, Zare, & Bakhtiyari, 2017).

The results in Table 1 also indicated a significant decrease in the concentration of hepcidin hormone in the blood serum of patients with β-Thalassemia major by 55% compared to healthy control of both sexes. Additionally, the results in Table 2 showed a significant decrease in the concentration of hepcidin hormone in the blood serum of males and females by 56 and 55% respectively compared to healthy control of both genders. The results in Table 3 also showed a significant decrease at a probability level of ($p \leqslant 0.05$) in the concentration of hepcidin in the blood serum of both males and females in all age groups, with the highest percentage decrease observed in females and males in the age group (11-15) years, by 63 and 59% respectively, compared to healthy control based on age groups and gender.

The results of this study are consistent with the findings of (Hasoon, Shani, & Radi, 2020; Smesam, Albuthabhak, Arjmand, Al-Hakeim, & Siadat, 2020; Au, Benjamin, & Wiśniewski, 2022), indicating a significant decrease in the concentration of hepcidin hormone in the blood serum of patients with β-Thalassemia major compared to healthy. The decrease in hepcidin hormone in β-Thalassemia and anaemia patients is attributed to hypoxia, which increases the production of ineffective red blood cells. A decrease in the hormone hepcidin leads to an increase in the amount of iron absorbed by the digestive system and from its stores in hepatic cells (Lee et al., 2018).

Hepcidin hormone is the main regulator of iron balance. The formation of ineffective red blood cells in patients with β-Thalassemia major, it leads to increased secretion of the hormone erythropoietin, which stimulates red blood cells to secrete growth differentiation factor 15 (GDF15), decrease in hepcidin hormone concentration leads to increased iron absorption. Therefore, estimating the concentration of hepcidin hormone is an important indicator for controlling iron balance (MuhammadJawad, Saeed, Mumtaz, Iram, & Mohsin, 2016; Taher & Saliba , 2017; Hasoon et al., 2020), Elevated iron level in the blood of patients with β-Thalassemia due to decreased hepcidin concentration, its lead to iron accumulation in the body, especially in the bone marrow, resulting in increased ferritin levels (Al-Hakeim & Al-Hakany, 2013). High iron levels in the patient population with β-Thalassemia major indicate iron diseases such as hemosiderosis and hemochromatosis (Finkenstedt et al., 2016). In response to acute anaemia, there is an increase in the production of erythropoietin hormone, which stimulates the bone marrow to produce RBCs. This hormone directly acts on liver cells to reduce the production of hepcidin hormone. Low concentrations of hepcidin and high concentrations of erythropoietin allow the release of stored iron, in addition to increasing the absorption of dietary iron to produce RBCs (Coffey & Ganz, 2018). The hepcidin hormone controls the concentration of iron in the blood directly by binding to the ferroprotein, it is a protein present on the membrane of RBCs, macrophages, intestinal cells, and hepatic cells (Lee et al., 2018).

Studies aimed to investigate a factor that links RBCs to iron regulation, leading to the identification of the erythroferrone factor, which is usually expressed significantly with erythropoietin, the red blood cell-stimulating hormone. It plays a regulatory role in RBCs in iron metabolism during oxygen deficiency or anaemia. Erythropoietin hormone stimulates the production of erythroferrone factor, which inhibits the

production of hepcidin hormone in the liver, leading to an increase in iron absorption in addition to the presence of large amounts of free iron available for the formation of RBCs (Eckardt et al., 2012).

The results in Table 1 showed a significant decrease in the concentration of growth hormone (GH) in the serum of patients with β-Thalassemia major, by 56% compared to healthy control of both sexes. The results in Table 2 also showed a significant decrease in GH concentration in male and female patients with this disease by 59 and 52%, respectively, compared to healthy control of both genders. The results in Table 3 also showed a significant decrease in GH concentration in the serum of males and females of all age groups studied and in both genders. The highest percentage of significant decrease was found in males and females in the age group (11-15) years, by 69 and 70%, respectively, compared to healthy control according to age groups and gender.

The results of the current study agreed with those of (Pincelli et al., 2011; Faiq, Hamabor, & Salih, 2022), who found a significant decrease in the concentration of growth hormone in the serum of patients with β-Thalassemia major compared to healthy individuals. This decrease is attributed to the effect of iron accumulation and deposition in the hypothalamic-pituitary region, leading to dysfunction of the pituitary gland and the area under the hypothalamus (Soliman et al., 1999; Wu et al., 2003; Pincelli et al., 2011). Patients with β-Thalassemia major are often short in stature, and the appearance of secondary sexual characteristics is delayed or may not appear at all, due to a deficiency in pituitary and gonadal hormones as a response to iron accumulation (Ohene-Frempong & Schwartz, 1980; Pincelli et al., 2011). The decrease in growth hormone in patients with β-Thalassemia major is also attributed to thyroid gland dysfunction, as studies have indicated that thyroid hormones play a strong role in regulating body growth (Smyczynska, Hilczer, Stawerska, & Lewinski, 2010).

Conclusion

It is concluded from this study that iron accumulation has a significant contribution to the increase in the concentration of both erythropoietin hormone and the decrease in hepcidin hormone, in addition to the decrease in growth hormone in the serum of patients with β-Thalassemia major compared to healthy control. This shows the extent to which thalassemia major affects some hormones, which indicates damage to the hypothalamus and pituitary gland.

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References

Al-Hakeim, H. K. A. H., & Al-Hakany, M. F. M. (2013). The effect of iron overload on the function of some endocrine glands in β-Thalassemia major patients. *Magazin of Al-Kufa University for Biology, 5*(2).

Ambachew, S., & Biadgo, B. (2017). Hepcidin in iron homeostasis: diagnostic and therapeutic implications in type 2 diabetes mellitus patients. *Acta Haematologica, 138*(4), 183-193. DOI: https://doi.org/10.1159/000481391

Amer, J., Dana, M., & Fibach, E. (2010). The antioxidant effect of erythropoietin on thalassemic blood cells. *Anemia*, 978710. DOI: https://doi.org/10.1155/2010/978710

Au, T. Y., Benjamin, S., & Wiśniewski, O. W. (2022). Is the role of hepcidin and erythroferrone in the pathogenesis of beta thalassemia the key to developing novel treatment strategies? *Thalassemia Reports, 12*(3), 123-134. DOI: https://doi.org/10.3390/thalassrep12030017

Bahadorimonfared, A., Alirezaei, A., Zare, E., & Bakhtiyari, M. (2017). Beyond hematopoietic property; administration of erythropoietin for nephroprotection. *Journal of Renal Injury Prevention, 6*(4), 292-296. DOI: https://doi.org/10.15171/jrip.2017.56

Brancaleoni, V., Di Pierro, E., Motta, I., & Cappellini, M. D. (2016). Laboratory diagnosis of thalassemia. *International Journal of Laboratory Hematology, 38*(S1), 32-40. DOI: https://doi.org/10.1111/ijlh.12527

Breymann, C., Fibach, E., Visca, E., Huettner, C., Huch, A., & Huch, R. (1999). Induction of fetal hemoglobin synthesis with recombinant human erythropoietin in anemic patients with heterozygous beta-

thalassemia during pregnancy. *The Journal of Maternal-Fetal Medicine, 8*(1), 1-7. DOI: https://doi.org/10.1002/(SICI)1520-6661(199901/02)8:1<1::AID-MFM1>3.0.CO;2-O

Caputo, M., Pigni, S., Agosti, E., Daffara, T., Ferrero, A., Filigheddu, N., & Prodam, F. (2021). Regulation of GH and GH Signaling by Nutrients. *Cells*, *10*(6), 1376.

Çetin, M., Ünal, Ş., Gümrük, F., Gürgey, A., & Altay, Ç. (2009). Serum erythropoietin levels in pediatric hematologic disorders and impact of recombinant human erythropoietin use. *Turkish Journal of Haematology, 26*(2), 72-76.

Chaisiripoomkere, W., Jootar, S., Chanjarunee, S., & Ungkanont, A. (1999). Serum erythropoietin levels in thalassemia major and intermedia. *Southeast Asian Journal of Tropical Medicine and Public Health, 30*(4), 786-788.

Clark, R. J., Tan, C. C., Preza, G. C., Nemeth, E., Ganz, T., & Craik, D. J. (2011). Understanding the structure/activity relationships of the iron regulatory peptide hepcidin. *Chemistry & Biology, 18*(3), 336-343. DOI: https://doi.org/10.1016/j.chembiol.2010.12.009

Coffey, R., & Ganz, T. (2018). Erythroferrone: an erythroid regulator of hepcidin and iron metabolism. *Hemasphere, 2*(2), e35. DOI: https://doi.org/10.1097/HS9.0000000000000035

Courselaud, B., Pigeon, C., Inoue, Y., Inoue, J., Gonzalez, F. J., Leroyer, P., ... Ilyin, G. (2002). C/EBPalpha regulates hepatic transcription of hepcidin, an antimicrobial peptide and regulator of iron metabolism. Cross-talk between C/EBP pathway and iron metabolism. *Journal of Biological Chemistry, 277*(43), 41163-41170. DOI: https://doi.org/10.1074/jbc.M202653200

De Sanctis, V. (2002). Growth and puberty and its management in thalassaemia. *Hormone Research, 58*(suppl. 1), 72-79. DOI: https://doi.org/10.1159/000064766

De Sanctis, V., Kattamis, C., Canatan, D., Soliman, A. T., Elsedfy, H., Karimi, M., ... Angastiniotis, M. (2017). β-thalassemia distribution in the old world: an ancient disease seen from a historical standpoint. *Mediterranean Journal of Hematology and Infectious Diseases, 9*(1), e2017018. DOI: https://doi.org/10.4084/MJHID.2017.018

Duchnowska, R., & Szczylik, C. (2003). Non-hematological role of erythropoietin. *Onkologia Polska, 6*(3), 109-112.

Eckardt, K.-U., Bärthlein, B., Baid-Agrawal, S., Beck, A., Busch, M., Eitner, F., ... Titze, S. (2012). The German chronic kidney disease (GCKD) study: design and methods. *Nephrology Dialysis Transplantation, 27*(4), 1454-1460. DOI: https://doi.org/10.1093/ndt/gfr456

Faiq, A. B., Hamabor, S. O., & Salih, M. A. H. (2022). Assessment of liver, thyroid gland and growth hormone functions in beta thalassemia major. *Iraqi Journal of Science, 63*(4), 1413-1422. DOI: https://doi.org/10.24996/ijs.2022.63.4.2

Finkenstedt, A., Krapf, S., Viveiros, A., Schäfer, B., Griesmacher, A., Vogel, W., & Zoller, H. (2016). The "iron score" is an meld-independent predictor of survival in patients with liver cirrhosis. *Journal of Hepatology, 64*(2), S443. DOI: https://doi.org/10.1016/s0168-8278(16)00731-5

Ganz, T. (2006). Hepcidin—a peptide hormone at the interface of innate immunity and iron metabolism. *Current Topics in Microbiology and Immunology, 306*, 183-198. DOI: https://doi.org/10.1007/3-540-29916-5_7

Gholamzadeh, R., Eskandari, M., Bigdeli, M. R., & Mostafavi, H. (2018). Erythropoietin pretreatment effect on blood glucose and its relationship with inflammatory factors after brain ischemic-reperfusion injury in rats. *Basic and Clinical Neuroscience, 9*(5), 347-356. DOI: https://doi.org/10.32598/bcn.9.5.347

Haase, V. H. (2013). Regulation of erythropoiesis by hypoxia-inducible factors. *Blood Reviews, 27*(1), 41-53. DOI: https://doi.org/10.1016/j.blre.2012.12.003

Hamed, O. M., Al-Taii, R. A., & Jankeer, M. H. (2021). Biochemical and genetic study in blood of β–Thalassaemia children in mosul city, Iraq. *Iraqi Journal of Science, 62*(8), 2501-2508. DOI: https://doi.org/10.24996/ijs.2021.62.8.2

Hasoon, I. G., Shani, W. S., & Radi, A. M. (2020). The association of hepcidin with some inflammatory markers in β-thalassemia major patients of Basrah Province. *Eurasian Journal of BioSciences, 14*, 7285-7289.

Hesham, M. A., El-Safy, U. R., El-Taweel, Y. A.-H., & Omar, M. M. H. (2022). Frequency of neurological manifestations in β-Thalassaemic patients in zagazig university hospitals. *The Egyptian Journal of Hospital Medicine, 86*, 391-397. DOI: https://doi.org/10.21608/EJHM.2022.212847

Hinton, P. R. (2004). Statistics explaned (2nd ed.). New York, NY: Routledge.

Huang, Y., Lei, Y., Liu, R., Liu, J., Yang, G., Xiang, Z., ... Lai, Y. (2019). Imbalance of erythropoiesis and iron metabolism in patients with thalassemia. *International Journal of Medical Sciences, 16*(2), 302-310. DOI: https://doi.org/10.7150/ijms.27829

Katavetin, P., Inagi, R., Miyata, T., Shao, J., Sassa, R., Adler, S., ... Nangaku, M. (2007). Erythropoietin induces heme oxygenase-1 expression and attenuates oxidative stress. *Biochemical and Biophysical Research Communications*, *359*(4), 928-934. DOI: https://doi.org/10.1016/j.bbrc.2007.05.207

Krohn, K., Haffner, D., Hügel, U., Himmele, R., Klaus, G., Mehls, O., & Schaefer, F. (2003). $1,25(OH)_2D_3$ and dihydrotestosterone interact to regulate proliferation and differentiation of epiphyseal chondrocytes. *Calcified Tissue International, 73*, 400-410. DOI: https://doi.org/10.1007/s00223-002-2160-9

Lee, N., Makani, J., Tluway, F., Makubi, A., Armitage, A. E., Pasricha, S.-R., ... Cox, S. E. (2018). Decreased Hepcidin levels are associated with low steady-state hemoglobin in children with sickle cell disease in Tanzania. *EBioMedicine, 34*, 158-164. DOI: https://doi.org/10.1016/j.ebiom.2018.07.024

Lin, H., Luo, X., Jin, B., Shi, H., & Gong, H. (2015). The effect of EPO gene overexpression on proliferation and migration of mouse bone marrow-derived mesenchymal stem cells. *Cell Biochemistry and Biophysics, 71*, 1365-1372. DOI: https://doi.org/10.1007/s12013-014-0358-x

Lundby, C., & Olsen, N. V. (2011). Effects of recombinant human erythropoietin in normal humans. *The Journal of Physiology, 589*(Pt. 6), 1265-1271. DOI: https://doi.org/10.1113/jphysiol.2010.195917

MuhammadJawad, I. A., Saeed, M. T., Mumtaz, G., Iram, S., & Mohsin, S. (2016). Hepcidin levels in multi transfused β thalassemia major patients. *Journal of Rawalpindi Medical College, 20*(3).

Nguyen, H., & Nguyen, H. (2017). AB023. Evaluation the outcome of β-thalassemia intermedia patients on hydroxyurea combined with erythropoietin at National Children's Hospital. *Annals of Translational Medicine, 5*(Suppl 2), AB023. DOI: https://doi.org/10.21037/atm.2017.s023

Ohene-Frempong, K., & Schwartz, E. (1980). Clinical features of thalassemia. *Pediatric Clinics of North America, 27*(2), 403-420. DOI: https://doi.org/10.1016/s0031-3955(16)33858-5

Pincelli, A. I., Masera, N., Tavecchia, L., Perotti, M., Perra, S., Mariani, R., ... Masera, G. (2011). GH deficiency in adult B-thalassemia major patients and its relationship with IGF-1 production. *Pediatric Endocrinology Reviews: PER, 8*(Suppl 2), 284-289.

Polat, C. (2021). Mutation analysis of beta-thalassemia major patients and their parents in diyarbakir Province, Turkey. *Dicle Tıp Dergisi, 48*(1), 47-54. DOI: https://doi.org/10.5798/dicletip.887407

Rivella, S. (2015). β-thalassemias: paradigmatic diseases for scientific discoveries and development of innovative therapies. *Haematologica,* 100(4), 418-430. DOI: https://doi.org/10.3324/haematol.2014.114827

Smesam, H. N. K., Albuthabhak, H. A. Q., Arjmand, S., Al-Hakeim, H. K., & Siadat, S. O. R. (2020). Evaluation of erythroferrone, hepcidin, and iron overload status in Iraqi transfusion-dependent β-thalassemia major patients. *Hemoglobin, 44*(4), 272-277. DOI: https://doi.org/10.1080/03630269.2020.1794888

Smyczynska, J., Hilczer, M., Stawerska, R., & Lewinski, A. (2010). Thyroid function in children with growth hormone (GH) deficiency during the initial phase of GH replacement therapy - clinical implications. *Thyroid Research, 3*(1), 2. DOI: https://doi.org/10.1186/1756-6614-3-2

Soliman, A. T., Abushahin, A., Abohezeima, K., Khalafallah, H., Adel, A., Elawwa, A., & Elmulla, N. (2011). Age related IGF-I changes and IGF-I generation in thalassemia major. *Pediatric Endocrinology Reviews, 8*(Suppl 2), 278-283.

Soliman, A. T., ElZalabany, M. M., Mazloum, Y., Bedair, S. M., Ragab, M. S., Rogol, A. D., & Ansari, B. M. (1999). Spontaneous and provoked growth hormone (GH) secretion and insulin-like growth factor I (IGF-I) concentration in patients with beta thalassaemia and delayed growth. *Journal of Tropical Pediatrics, 45*(6), 327-337. DOI: https://doi.org/10.1093/tropej/45.6.327

Souma, T., Suzuki, N., & Yamamoto, M. (2015). Renal erythropoietin-producing cells in health and disease. *Frontiers in Physiology, 6*, 167. DOI: https://doi.org/10.3389/fphys.2015.00167

Taher, A. T., & Saliba, A. N. (2017). Iron overload in thalassemia: different organs at different rates. *The American Society of Hematology Education Program*, (1), 265-271. DOI: https://doi.org/10.1182/asheducation-2017.1.265

Wu, K. H., Tsai, F. J., & Peng, C. T. (2003). Growth hormone (GH) deficiency in patients with beta-thalassemia major and the efficacy of recombinant GH treatment. *Annals of Hematology, 82*(10), 637-640. DOI: https://doi.org/10.1007/s00277-003-0712-3