HAYATI
Journal of Biosciences

Vol. 32 No. 6, November 2025 1518-1528
DOI:10.4308/hjb.32.6.1518-1528
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Check for updates

OPEN ACCESS

Comparative Analysis of the Age, Gender, and Ferritin Levels with Hepcidin in Iraqi Patients Suffering Beta Thalassemia (Major and Intermedia)

Istabraq A. Al-Husseiny[1*], Essam F. Al-Jumaili[2]

[1]*Tropical Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq*
[2]*Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq*

ARTICLE INFO

Article history:
Received September 1, 2024
Received in revised form June 18, 2025
Accepted July 25, 2025
Available Online August 27, 2025

KEYWORDS:
Beta Thalassemia Major,
Beta Thalassemia Intermedia,
Hb,
Ferritin,
hepcidin

Copyright (c) 2025@ author(s).

ABSTRACT

Beta thalassemia is a hereditary, autosomal recessive blood condition. The present investigation aims to assess the relationship between ferritin and hepcidin serum levels, age, and gender in patients with β-thalassemia major (βTM) and β-thalassemia intermedia (βTI). Two groups of 100 patients, including 39 females and 61 males, were identified: 73 patients had (βTM), and 27 had range spans. The age range spans from 1 to 46 years. The patients were registered at the Al-Krama Teaching Hospital, Hereditary Blood Disorder Center, Baghdad, between June 2023 and April 2024. The study's control group consisted of 50 healthy individuals in similar age groups. Human ELISA kits were used to measure the amounts of serum ferritin and hepcidin. The mean age of the βTM and βTI patients differed significantly from that of the control groups (p-value 0.001). Gender-wise, males were more affected than females in the βTM and βTI groups. The Hb concentration was much lower in the patient's blood samples. The Ferritin with hepcidin concentrations in the serum of βTM and βTI showed a significant difference at the 0.001 level. The control had the lowest value. The current study's findings demonstrated elevated blood levels of hepcidin and Ferritin in the βTM and βTI groups, with the βTI group showing noticeably higher levels.

1. Introduction

Alpha and beta thalassemia are caused by four genes that code for alpha and beta globin. Thus, the number of these genes that are absent or faulty determines the severity of alpha and beta-thalassemia (Abbas *et al.* 2020). β-thalassemia is one of the most widely distributed single-gene diseases worldwide. It has several defects in hemoglobin synthesis, including those resulting from a decreased yield of β-globin protein. In the β-globin gene, five distinct mutations were identified, which were categorized into five separate mutations (Hamed *et al.* 2021). According to Musallam *et al.* (2012), there are three forms of β-thalassemia based on clinical characteristics: β-thalassemia major (βTM), β-thalassemia intermediate (βTI), and β-thalassemia minor. The symptoms of thalassemia major include severe anaemia, bone marrow enlargement, and hypertrophy. Patients with intermediate thalassemia require frequent blood transfusions to maintain a normal life (Meri *et al.* 2022). This disorder lies between minor and major kinds and allows patients to lead normal lives except for the sporadic requirement for blood transfusion during pregnancy and illness (Baqer & Al-Humairi 2022). A single-chain shortage causes minor thalassemia; although the patient has no symptoms, routine blood tests reveal basic anaemia (Baird *et al.* 2022). Patients' treatment options are based on the severity of their thalassemia. Blood transfusions are the standard treatment for thalassemia (Mahmoud *et al.* 2024). The production of hemopoietic growth factors is typically found to be a controlled cascade reaction (Khalaf *et al.* 2022) that enables flexible and comprehensive responses to various

* Corresponding Author
E-mail Address: istabraq.hussein@sc.uobaghdad.edu.iq

hemopoietic stressors. On the other hand, erythrocytes undergo intramedullary death instead of proliferating from erythroid progenitor cells.

In individuals with β-thalassemia intermedia, inadequate erythropoiesis increases red blood cell production (Rivella 2012). The main factor contributing to morbidity and death in β-thalassemia, whether or not transfusion need is present, is hyperuricemia. Since serum ferritin is thought to underestimate hyperironemia, particularly in β-thalassemia intermediate patients, the number of patients with iron excess may be larger. The hepatic peptide hormone hepcidin is established as the main regulator of iron homeostasis. The release of stored iron from hepatocytes, the release of recycled iron from macrophages, and dietary absorption are the three main sources of iron that are inhibited by the tiny peptide hormone hepcidin. Hepatocytes transform pro-hepcidin, which is produced, into bioactive hepcidin, which is then released into the bloodstream and eliminated by the kidneys (Rumjon 2019).

In Iraq, thalassemia is a frequent autosomal recessive illness with an incidence of 35.7 per 100,000 people (Nemeth 2010). The three primary causes of thalassemia are venous thrombosis, biliary obstruction, and chronic iron excess. These variables may lead to cirrhosis, fibrosis, and, in cases of thalassemia, hepatocellular carcinoma (Majid *et al.* 2021). Beta thalassemia major is a severe and life-threatening condition that, if left untreated, can result in early mortality and has significant health implications. Given its profound impact, it is essential to conduct a comparative analysis of age, gender, and ferritin levels of hepcidin among Iraqi patients with beta-thalassemia (both major and intermediate). This analysis aims to deepen the understanding of how these factors correlate with disease severity. Furthermore, the study seeks to identify potential biomarkers for early detection, assess the effects of iron overload on hepcidin regulation, and explore personalized treatment strategies to enhance patient outcomes. By addressing these objectives, the research aims to contribute to the improvement of clinical management and therapeutic approaches for thalassemia patients in Iraq.

2. Materials and Methods

A total of 100 individuals with β-thalassemia (39 females and 61 males) were divided into two categories based on the type of β-thalassemia they had: 73 patients with β-thalassemia major (β-thalassemia major, βTM) and 27 patients with β-thalassemia intermedia (β-thalassemia intermedia, βTI). Blood samples were taken from each group. After being recognized by experts, they received continuous iron chelator therapy. Three age groups were created: 1–14 years old, 15–30 years old, and 31–46 years old. Between June 2023 and April 2024, the patients were enrolled at the Al-Krama Teaching Hospital, Hereditary Blood Disorder Center, Baghdad, which is part of the Ministry of Health in Iraq. Each research participant gave a signed written agreement, and a specific questionnaire form was used to gather subject data. As an instance of control, 50 healthy participants identical in age range—35 females and 15 males—were also included in the study. Data were gathered through in-depth clinical examinations and comprehensive history gathering during patient interviews. The spleen was not surgically removed from any individuals included in this research. Individuals with beta thalassemia minor, hepatitis B, or these conditions were not allowed to participate in this study. For this study, participants in the control group who appeared to be in good health and had no known hereditary or chronic medical conditions were selected. This research was conducted at the Iraqi Hereditary Company, Al-Harthiya, Baghdad.

2.1. Ethical Approval

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before a sample was taken. The study protocol, subject information, and consent form were reviewed and approved by a local ethics committee, as documented in document number H T 2342 (dated 21/5/2023), to obtain this approval.

2.2. Sample Acquisition

From each study group, five milliliters of venous blood were taken. The blood was separated into three milliliters and preserved in Ethylenediamine Tetraacetic Acid (EDTA) tubes. Two milliliters were transferred to a gel tube, and the tube was centrifuged for five minutes at 3,000 rpm to extract serum. The tube was then stored in a freezer at -20°C with the collected serum until it was needed.

2.3. Assessment of Serum Ferritin and Hepcidin Levels

The blood serum was used to evaluate the levels of many biochemical parameters. Human ELISA hepcidin kits (Bioassay Technology Laboratory, China;

E 1019Hu) and Human Enzyme-Linked Immunosorbent Assay (ELISA) ferritin kits (Bioassay Technology Laboratory, China; E 1702Hu) were used to assess serum ferritin and hepcidin levels. The quantities in the serum were determined using established spectrophotometric methods in accordance with the provided criteria.

2.4. Statistical Analysis

To analyze the statistics and determine how various factors affected the research parameters, the IBM SPSS Statistics 26 application was utilized. To compare means substantially, one-way ANOVA and the T-test were employed. To compare percentages (with probabilities of 0.05 and 0.01), the chi-square test was employed. Odds ratios (OR) with Cornfield 95% confidence intervals (CIs) were computed by logistic regression using the same software used in this research. The figures in this study were created using the GraphPad Prism 9 application. To find the genotyping, WINPEPI and the SPSS application were utilized (Luaibi & Mohammed 2024).

3. Results

3.1. The Population Size of Patients with βTM and βTI

One hundred thalassemia patients out of 1300 patients registered in the "Hereditary Blood Disorder Center at Al-Karama Teaching Hospital" were included in the research (Table 1), 73 patients out of 954 patients in the centre had β-thalassemia major (βTM), and 27 patients out of 346 patients of both sexes had β-thalassemia intermedia (βTI). The patients' ages ranged from 1 to 46 years, with 50 healthy individuals of both genders within the same age range serving as a control group.

Table 1. Distribution of study sample size relative to hospital population in β-thalassemia major (βTM) and β-thalassemia intermedia (βTI) patients

Groups	Population size (hospital)	Sample size of patients (study)
Btm	954	73
βTI	346	27
Total	1300	100

3.2. Distribution of (βTM and βTI) and Control According to Age

When comparing the two groups, βTM and βTI, to the control group, a highly significant statistical difference was observed (p-value = 0.001). The present study examines the percentage distribution of βTM and βTI thalassemia patients and controls across three distinct age groups: 1-14 years, 15-30 years, and 31-46 years. Table 2 shows that the mean age in the three groups, βTM, βTI, and control, is not significantly different.

According to (Table 3), the first age group of patients with β-thalassemia major showed substantial increases of 22.7% when compared to the healthy control group and patients with β-thalassemia intermediate.

3.3. Distribution of βTM, βTI, and Control According to Gender

Beta thalassemia major affects 46 (30.7%) males and 27 (18.0%) females. Furthermore, 15 (10.0%) males and 12 (8.0%) females have β-thalassemia

Table 3. Distribution of βTM, βTI, and control groups by age

		Age. groups			Total
		1-14	15-30	31-46	
Groups	βTM	34 22.7%	31 20.7%	8 5.3%	73
	βTI	11 7.3%	14 9.3%	2 1.3%	27
	Control	9 6.0%	24 16.0%	17 11.3%	50
p-value		0.001**			

Table 2. Comparison between (βTM and βTI) and the control groups in age

Groups	Age. groups	Mean	Std. deviation	Std. error of mean	p-value
βTM	1-14	9.1765[c]	3.74547	.64234	0.001**
	15-30	22.4839[b]	5.03899	.90503	
	31-46	36.2500[a]	4.94975	1.75000	
βTI	1-14	10.8182[c]	3.40053	1.02530	0.001**
	15-30	20.5000[b]	4.58677	1.22587	
	31-46	40.0000[a]	1.41421	1.00000	
Control	1-14	10.6667[c]	2.73861	.91287	0.001**
	15-30	21.0417[b]	5.27899	1.07757	
	31-46	38.5882[a]	5.74520	1.39342	

intermedia. Thirty-five female controls (23.3%) and 15 male controls (10.0%) are also included. Overall, (Table 4) shows a highly significant difference at the 0.001 level.

3.4. Association between (βTM, βTI) and Control According to Hemoglobin

The hemoglobin (Hb) level is the most apparent difference between the control and both β-thalassemia (Major and Intermedia) groups. The hemoglobin levels of individuals with β-thalassemia (βTM and βTI) were 7.94±1.465 gm/L and 8.30±1.278 gm/L, respectively, as shown in (Table 5). In contrast, the mean hemoglobin level of the control group was 12.67±0.866 gm/L. The averages of all the measured parameters, hepcidin (HEP), Ferritin (FER), and Hemoglobin (Hb), varied significantly (p-value 0.001) between the control groups and the βTM and βTI groups. The results demonstrated that, compared to the control group depicted in Figure 1, the Hb concentration levels in the samples of the (βTM and βTI) patients were significantly lower.

Table 4. Comparison of the genders of the (βTM and βTI) and control groups

		Gender		Total
		Male	Female	
Groups	βTM	46 30.7%	27 18.0%	73 48.7%
	βTI	15 10.0%	12 8.0%	27 18.0%
	Control	15 10.0%	35 23.3%	50 33.3%
p-value		0.001**		

Table 5. Comparison of Hb level, FER, and HEP between (βTM and βTI) and control groups

Groups		Hb	FER (ng/ml)	HEP (ng/ml)
βTM	Mean	7.94[b]	187.39[a]	2096.27[a]
	Std. deviation	1.465	89.76	1010.15
	Std. error of mean	.366	13.689	154.047
Control	Std. deviation	8.30[b]	202.32[a]	2297.03[a]
	Std. error of mean	1.278	61.987	758.885
	Mean	.426	14.610	178.871
p-value	Std. Deviation	12.67[a]	83.95[b]	907.72[b]
		.289	7.610	41.74
		0.001**	0.001**	0.001**

3.5. Association between (βTM, βTI) and Control According to Ferritin

Table 5 and Figure 2 show a significant difference at the 0.001 level between the ferritin concentrations for major (187.39 ng/mL) and intermediate (202.32 ng/mL) β-thalassemia. The control group has the lowest value, at 83.95 ng/mL. Our findings demonstrated that patients' ferritin concentrations were higher than those of controls, and their Hb concentrations were lower. Our analysis indicates that the average blood ferritin concentration was 2767.52 ng/mL.

The current study's findings showed that the levels of Ferritin were abnormally high in the βTM and βTI groups (187.39 and 202.32 ng/mL, respectively), suggesting that excessive iron intake is a significant problem for patients with βTM and βTI.

The connection between major and intermediate β-thalassemia concentrations of Ferritin and optical density (OD) provided by ELISA results. Figure 3 displays our findings regarding the relationship between OD and ferritin concentration ($R^2 = 0.9799$) and the corresponding equation ($y = 0.0077x$).

3.6 Association between βTM, βTI, and Control According to Hepcidin

In Table 6, hepcidin concentrations for β-Thalassemia major (2096.27 ng/mL) and intermedia

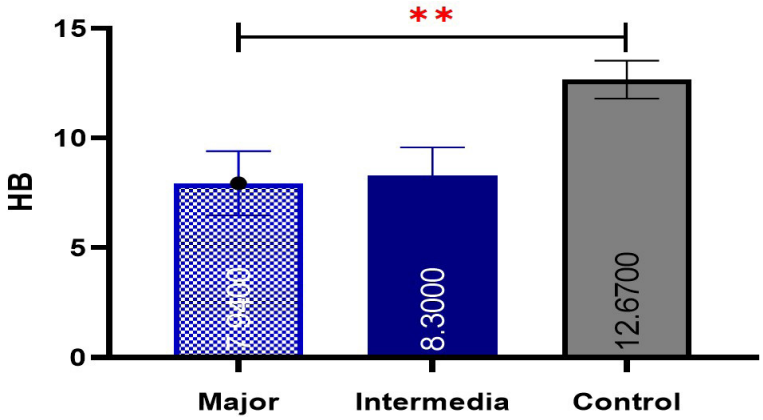

Figure 1. Correlation between control, intermediate forms of β-thalassemia, and hemoglobin

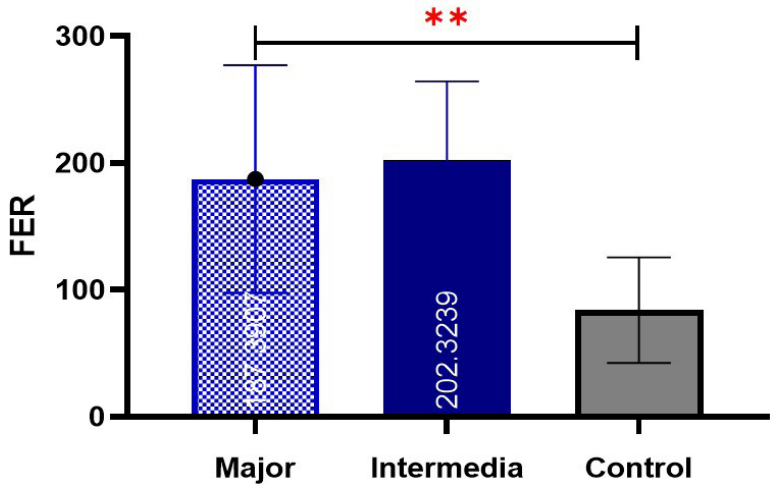

Figure 2. Relationship between ferritin serum, major and intermediate forms of β-thalassemia, and control

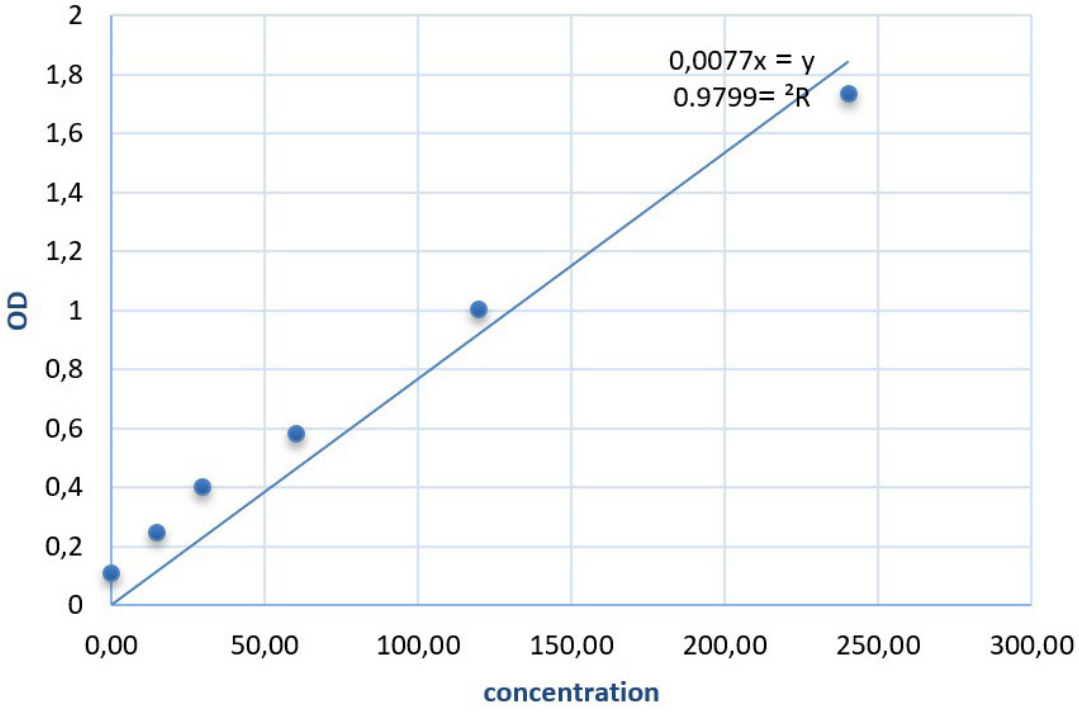

Figure 3. The relationship between OD and the concentration of serum ferritin, major and intermediate β-thalassemia

Table 6. The concentration of hepcidin in control, major, and intermediate β-thalassemia

Groups	Mean ± SE hepcidin (ng/ml)
βTM	2096.27±154.047[a]
βTI	2297.03±178.871[a]
Control	907.72±41.74[b]
LSD value	374.051**
P-value	0.001**

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

(2297.03 ng/mL) show a highly significant difference at the 0.001 level. The control group's result was the lowest, at 907.72 ng/mL. The present study found that individuals with β-thalassemia major and intermedia had higher hepcidin blood levels (2096.27 ng/mL and 2297.03 ng/mL, respectively).

3.7. The Connection between Optical Density (OD) and Hepcidin Concentration Provided by ELISA Results in Major and Intermediate β-Thalassemia

Figure 4 displays our findings regarding the relationship between OD and hepcidin concentration (R^2 = 0.9745) and the corresponding linear equation (y = 0.0006x) which indicates that 97.45% of the variation in OD can be explained by changes in hepcidin concentration, also demonstrating a very strong linear relationship and (y = 0.0006x): that shows for every 1 unit increase in hepcidin concentration, the OD increases by 0.0006 units.

3.8. The Receiver Operating Characteristic (ROC) Curve Data of Ferritin and Hepcidin

Receiver operating characteristic (ROC) curves were applied in our research to evaluate the discriminative diagnostic accuracy, sensitivity, and specificity of different test variables, and cut-off values were determined for each diagnostic test accordingly. The results of the ROC curve data of Ferritin and hepcidin are shown in Table 7.

A receiver operating characteristic curve of Ferritin (Figure 5) showed that 100.2600 was the threshold for Ferritin between sensitivity (80) and specificity (90). The result revealed that the curve's area under Ferritin was 0.85. When the area under the curve's value equals 0.001 or higher, it is perfect for anticipating disease prognosis.

Receiver operating characteristic curve of the hepcidin (Figure 6) showed that 1334.1700 was the threshold for hepcidin between sensitivity (72) and specificity (100). The result revealed that the area under the curve for hepcidin was 0.87. When the area under the curve's value equals 0.001 or higher, it means the model is perfect for predicting disease prognosis.

Combined ROC curve analysis of the Ferritin and hepcidin. Results recorded Hep's best performance characteristics, showing the highest sensitivity and specificity compared to FER. As shown in Figure 7.

4. Discussion

The present outcomes corroborate those of Qurat-ul-Ain *et al.* (2011), who found that βTM predominates over βTI. In other words, β-thalassemia, particularly β-thalassemia intermedia (βTI), is more common among older adults. This outcome concurs with that

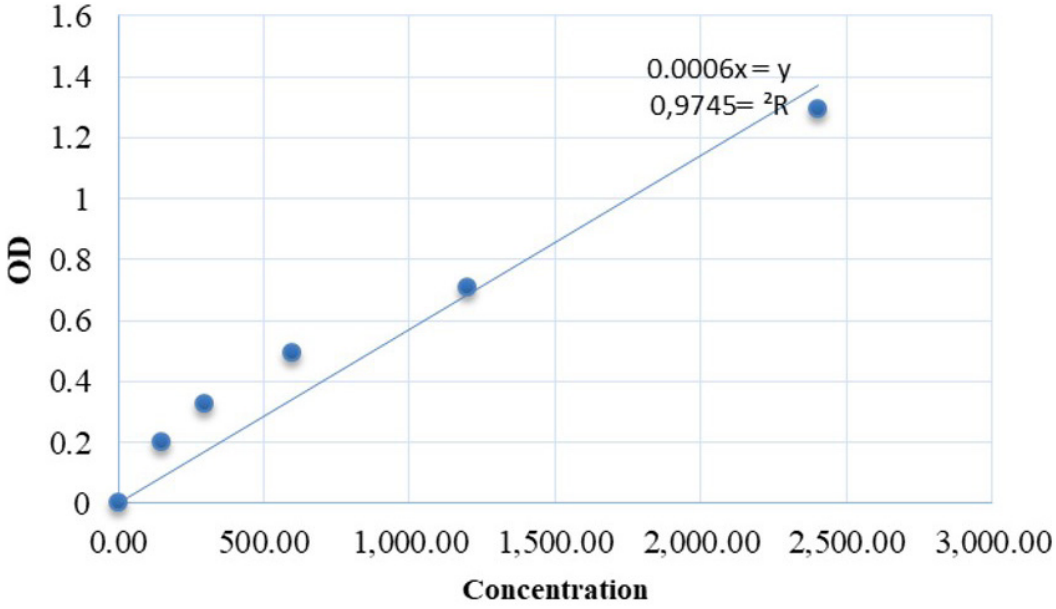

Figure 4. The relationship between OD and the concentration of serum hepcidin, major and intermediate β-thalassemia

Table 7. The receiver operating characteristic curve data of ferritin and hepcidin

Parameters	AUC	Explanation	P value	The best cut off	Sensitivity %	Specificity %
FER	0.85	Very good	0.001	100.2600	80	90
HEP	0.87	Very good	0.001	1334.1700	72	100

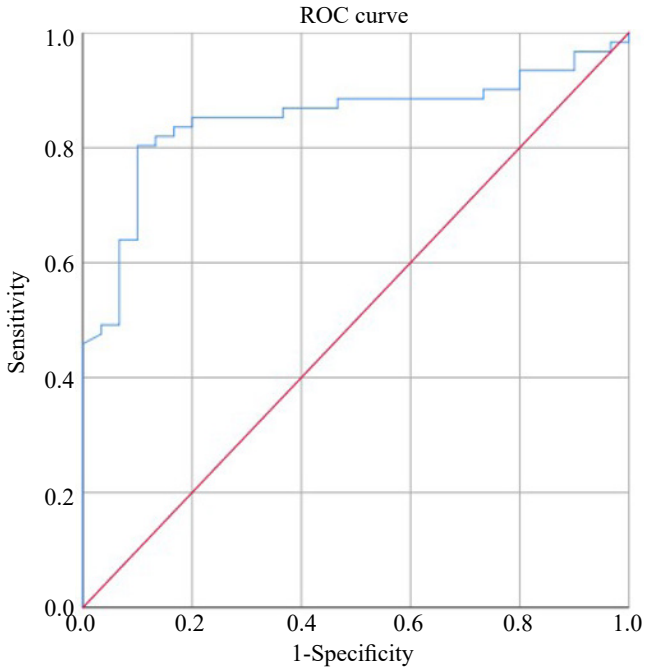

Figure 5. Receiver operating characteristic curve of the ferritin

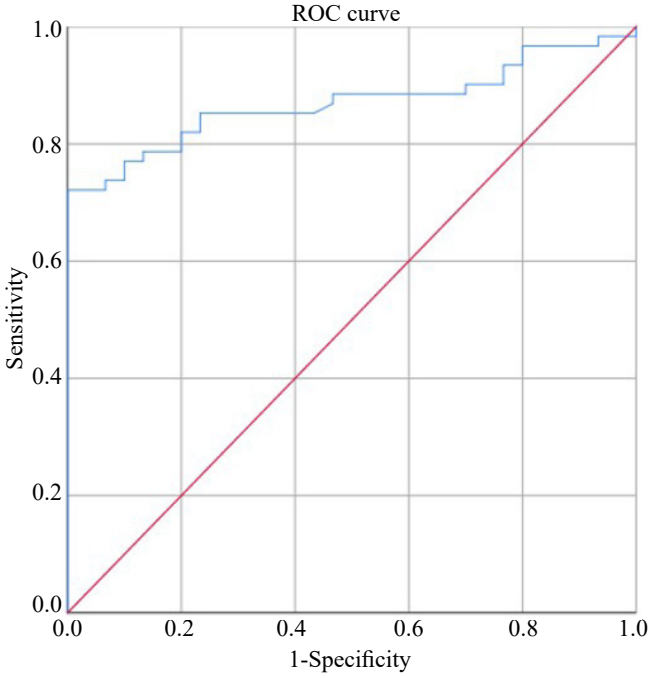

Figure 6. Receiver operating characteristic curve of the hepcidin

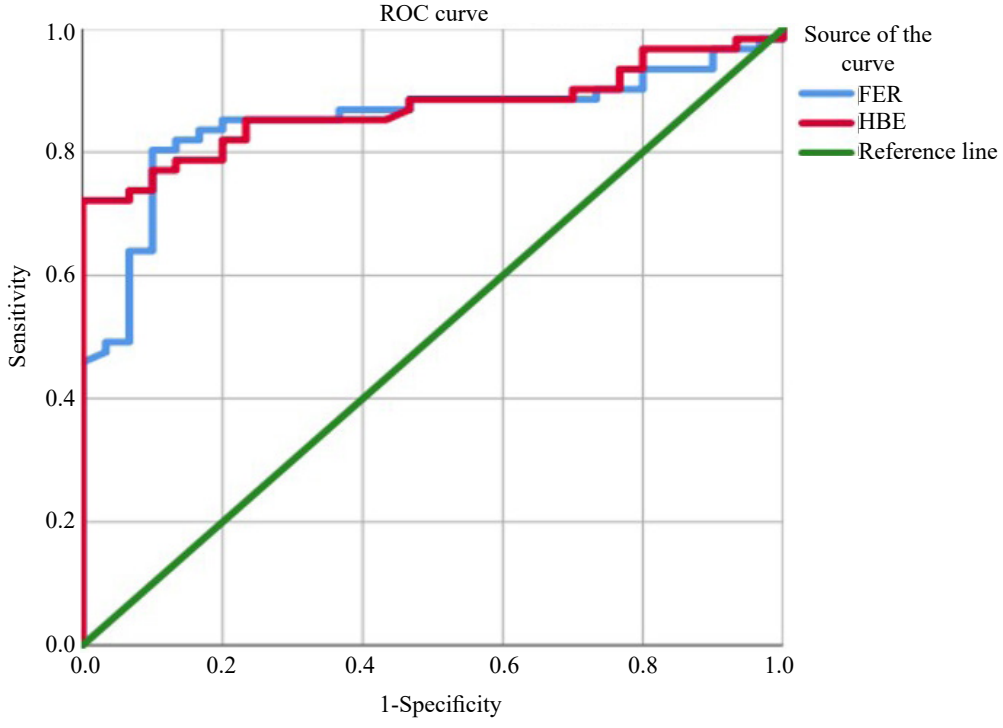

Figure 7. Combined ROC curve analysis of ferritin and hepcidin

of Al-Ali and Faraj (2016). Thalassemia was found to be more common among patients aged 1-14 years. This age-specific distribution may be attributed to several underlying factors, including genetic predisposition, early clinical manifestation of the disease, and the effectiveness of diagnostic screening protocols implemented during childhood. Thalassemia, being a hereditary hemoglobinopathy, often presents symptoms such as anaemia, jaundice, and growth retardation within the first few years of life, which likely contributes to its early detection in this age group. According to Hashim *et al.* (2020), the data collected suggested significant variations in the type of thalassemia between age groups, as determined by the Chi-square test (Faranoush *et al.* 2023).

Additionally, our data indicated that children between 1 and 14 had a higher prevalence of β-thalassemia major. On the other hand, Khalaf and Al-Saadi (2022) investigation in Iraq, which involved individuals between the ages of 11 and 18, produced similar findings. The discrepancy can be attributed to the overall number of samples gathered over a period of years; nonetheless. However, individuals with thalassemia major who were older than 18 years old were discovered (Luaibi & Mohammed 2023).

Moreover, the gender-related findings in our study differed from those reported by Al-Ali and Al-Musawi (2022). Discrepancies in clinical outcomes among patients, influenced by factors such as gender, race, and transfusion frequency, may explain the variations between our results and those of other studies. This result aligns with the research conducted by Al-Ali and Faraj (2016), which explored gender differences in β-thalassemia among Iraqi patients. Their findings indicated that males were more affected than females in both the β-thalassemia major (βTM) and β-thalassemia intermedia (βTI) groups, a trend also supported by the results of Qurat-ul-Ain *et al.* (2011). Lucarelli *et al.* (1996) state that β-thalassemia is an autosomal recessive disorder caused by mutations in the β-globin gene, located on chromosome 11, rather than a gender-related illness. Because a gender characteristic does not influence β-thalassemia, it affects both sexes equally. There is no correlation between gender and sickness, as the genes that produce the peptide chains that make up Hemoglobin are located on somatic chromosomes (Hashim *et al.* 2020).

The results demonstrated a significant decrease in Hemoglobin (Hb) concentration levels in patients with β-thalassemia major (βTM) and β-thalassemia intermedia (βTI) compared to the control group. This finding is consistent with the pathophysiology of β-thalassemia. The lower Hb levels in βTM and βTI patients highlight the severity of the condition, as these individuals often experience impaired red blood cell production and increased hemolysis. The significant difference in Hb concentration between the patient groups and the control group underscores the clinical impact of β-thalassemia on oxygen-carrying capacity and overall health. These results align with previous studies that have documented reduced Hb levels in thalassemia patients due to ineffective erythropoiesis and ongoing hemolytic processes. This result is consistent with the findings of Sundaresan *et al.* (2023). The findings from Ali *et al.* (2021) in Baghdad, Al-Dedah *et al.* (2018) in Karbala, and Al-Zuhairy *et al.* (2021) in Misan corroborated our findings, demonstrating that patients' ferritin concentrations were higher than those of controls, and their Hb concentrations were lower. Mishra and Tiwari (2013) discovered that anomalously elevated ferritin levels were observed in 87.4% of patients with severe β-thalassemia.

The current study's findings showed that the levels of Ferritin were abnormally high in the βTM and βTI groups, suggesting that excessive iron intake is a significant problem for patients with βTM and βTI. Blood transfusions may result in iron overload. The effects of iron excess after red blood cell transfusion on the severity of the illness and inefficient and extramedullary erythropoiesis (Shawkat & Jwaid 2019). Reports that serum ferritin levels offer an additional prognostic evaluation for predicting various clinical outcomes in individuals with β-thalassemia (Shah *et al.* 2022).

The iron content of Ferritin has increased in recent investigations, suggesting that this is the primary source of elevated Ferritin Levels. Unstable hemoglobin variations, especially those with β-thalassemia characteristics, cause congenital hemolytic anemia. The most accurate single measure of total body iron is serum ferritin. Patients with iron excess have historically experienced sickness in beta-thalassemia patients. The cause of high levels of serum ferritin, which in turn depends on the iron overload brought on by frequent blood transfusions. Furthermore, due to oxidative stress and iron's direct toxicity to liver cells (Luaibi & Mohammed 2024).

β-thalassemia patients with iron overload have been shown in earlier investigations to have decreased blood hepcidin levels (Tantiworawit *et al.* 2021). Hepcidin, a

key regulator of iron homeostasis, is typically suppressed in iron overload conditions to enhance iron absorption and release from storage. However, the increased hepcidin levels observed in β-thalassemia patients may seem counterintuitive given the chronic iron overload commonly associated with the disease, particularly in those receiving regular blood transfusions. However, the present study found that people with β-thalassemia major and intermedia possessed higher blood levels of hepcidin. This elevation in hepcidin levels could be attributed to several factors. First, chronic inflammation, which is often present in β-thalassemia patients due to repeated transfusions and hemolysis, may stimulate hepcidin production. Inflammatory cytokines, such as interleukin-6 (IL-6), are known to upregulate hepcidin expression. Second, ineffective erythropoiesis, a hallmark of β-thalassemia, may also play a role. While erythropoietic activity typically suppresses hepcidin to meet the increased demand for iron in red blood cell production, the dysregulation of this process in β-thalassemia could lead to abnormal hepcidin levels, matched the results of (Ismail *et al.* 2019), who discovered that there was a significant difference between mean Ferritin and hepcidin in individuals with βTM and βTI ($P = 0.042$, $P<0.001$). Both groups were shown to have high blood levels of hepcidin in the current analysis; however, those with β-thalassemia intermedia had much higher levels than those with β-thalassemia major. Hepcidin was higher in β-thalassemia patients than in controls ($p = 0.001$). Hepcidin and ferritin levels were greater in the βTI than in the βTM (0.001). In individuals with β-thalassemia major, hepcidin can be used as a logical medicinal agent to treat the condition and can be a valuable marker to determine the degree of iron overload, especially in the heart (Ayatollahi *et al.* 2020).

According to Huang *et al.* (2019), hepcidin levels were favorably correlated with serum iron, Hemoglobin, and Ferritin, but negatively correlated with erythropoiesis (Youssry *et al.* 2024). However, hepcidin may be more impacted by erythropoiesis or iron chelation therapy than iron storage, as suggested by the findings of other researchers (Zarghamian *et al.* 2020), (Tantiworawit *et al.* 2024), (Zaman and Ibrahim 2022), and (Al-Omari & Takruri 2022), who found no significant correlation between hepcidin and serum ferritin, hemoglobin, or serum free iron.

The ELISA results reveal strong correlations between optical density (OD) and the concentrations of two key biomarkers—Ferritin and hepcidin—in patients with primary and intermediate β-thalassemia. These findings are significant for understanding iron metabolism and its dysregulation in β-thalassemia. OD is a measure of light absorbance by a sample, which is proportional to the concentration of the target molecule (Ferritin or hepcidin). $R^2 = 0.9799$ in the ferritin concentration curve (Figure 3) indicates that 97.99% of the variation in OD can be explained by changes in ferritin concentration, demonstrating an extremely strong linear relationship. In contrast, the regression equation ($y = 0.0077x$) indicates that for every 1 unit increase in ferritin concentration, the OD increases by 0.0077 units. Also, the Hepcidin Concentration result curve (Figure 4) when the value ($R^2 = 0.9745$), which indicates that changes in hepcidin concentration can explain 97.45% of the variation in OD, also demonstrates a very strong linear relationship and ($y = 0.0006x$): that shows for every 1 unit increase in hepcidin concentration, the OD increases by 0.0006 units. This result confirms a direct proportionality between hepcidin levels and OD, though the slope is much smaller compared to Ferritin. These findings highlight the reliability of ELISA for quantifying these biomarkers, which are critical for understanding and managing iron dysregulation in β-thalassemia. The dual measurement of Ferritin and hepcidin provides valuable insights into iron storage and regulation, aiding in the clinical management of these patients. Tantiworawit *et al.*'s findings are contradictory (2021). In the current study, the Ferritin and hepcidin levels of beta-thalassemia patients were evaluated using the ROC curve to demonstrate that either one of these parameters is a suitable indicator for diagnosis and prognosis prediction in patients. In the present investigation, it was observed that the accuracy of hepcidin was superior to that of Ferritin, as it exhibited higher diagnostic accuracy in identifying patients with beta-thalassemia.

In conclusion, the findings demonstrate that β-thalassemia major (βTM) is more prevalent than β-thalassemia intermedia (βTI), especially in younger age groups (1–14 years). However, a significant prevalence is also observed in older patients (over 18 years). Gender differences were noted, with males more affected than females, despite β-thalassemia being an autosomal recessive disorder unrelated to gender. Patients with βTM and βTI exhibited significantly lower Hemoglobin (Hb) levels and elevated ferritin concentrations, indicative of iron overload due to frequent transfusions and ineffective erythropoiesis.

Hepcidin levels, a key regulator of iron metabolism, were also elevated, particularly in βTI, highlighting its potential as a therapeutic target and marker for iron overload severity. ELISA results revealed strong linear relationships between optical density (OD) and Ferritin and hepcidin, confirming ELISA's reliability for quantifying these biomarkers. ROC curve analysis further identified hepcidin as having superior diagnostic accuracy over Ferritin in identifying β-thalassemia patients. These findings emphasize the importance of dual ferritin and hepcidin measurements in understanding iron dysregulation and improving clinical management in β-thalassemia.

References

Abbas, A.H., Hassan, I.B., Al-Malkey, M.K., Mohammed-Saeed, S.W., 2020. Genetic polymorphisms frequency of vitamin D receptor gene rs7975232 and rs731236 in Iraqi thalassemic patients and healthy controls compared to Arabian healthy populations. *Meta Gene*. 25, 100723. https://doi.org/10.1016/j.mgene.2020.100723

Al-Ali, S., Al-Musawi, R., 2022. The relevance of rs34598529 SNP of HBB gene among β-thalassemic patients dependent on blood transfusions in Thi-Qar Governate. *Iraqi J Biotechnol*. 21, 668-676.

Al-Ali, Z., Faraj, S.H., 2016. Prevalence of β-thalassemia patients in Missan Province. *GJBAHS*. 5, 68-70.

Al-Dedah, R.M., Al-wazni, W.S., Al-Ghanimi, H.H., Abduallah, F., 2018. Biochemical and hematological study with the appreciation of some immunological parameters in thalassemia patients at Kerbala Province. *Journal of Pure & Applied Microbiology*. 12, 1965-1973. https://doi.org/10.22207/JPAM.12.4.33

Al-Omari, D.A., Takruri, H.R., 2022. Status of hepcidin, superoxide dismutase, zinc, and copper in β-thalassemia. *JSSFN*. 15, 11-18.

Al-Zuhairy, S.H., Darweesh, M.A., Othman, M.A., 2021. Relation of serum ferritin level with serum hepcidin and fucose levels in children with β-thalassemia major. *Hemoglobin*. 45, 69-73. https://doi.org/10.1080/03630269.2021.1898419

Ali, S., Mumtaz, S., Shakir, H.A., Khan, M., Tahir, H.M., Mumtaz, S., Mughal, T.A., Hassan, A., Kazmi, S.A.R., Sadia, 2021. Current status of beta-thalassemia and its treatment strategies. *Molecular Genetics & Genomic Medicine*. 9, e1788. https://doi.org/10.1002/mgg3.1788

Ayatollahi, H., Mousavi Nezhad, S.F., Talebpour, A., Badiei, Z., Nezami, H., 2020. Relation of hepcidin gene expression in blood mononuclear cells with iron overload severity among β-thalassemia major patients. Molecular Biology Reports, 47, 9353-9359. https://doi.org/10.1007/s11033-020-06012-2

Baird, D.C., Batten, S.H., Sparks, S.K., 2022. Alpha-and beta-thalassemia: rapid evidence review. *American Family Physician*. 105, 272-280.

Baqer, O.M., Al-Humairi, A.K., 2022. Knowledge, attitude, and practice of junior doctors about thalassemia in Babylon Province. *Medical Journal of Babylon*. 19, 162-168. https://doi.org/10.4103/MJBL.MJBL_75_21

Faranoush, M., Faranoush, P., Heydari, I., Foroughi-Gilvaee, M. R., Azarkeivan, A., Parsai Kia, A., Sadighnia, N., Elahinia, A., Zandi, A., Rezvany, M.R., 2023. Complications in patients with transfusion dependent thalassemia: a descriptive cross-sectional study. *Health Science Reports*. 6, e1624. https://doi.org/10.1002/hsr2.1624

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, 2501-2508.

Hashim, N.A., Abdullah, Y.J., Ibadi, H.A., 2020. Evolution of some biochemical and hematological parameters of thalassemia patients in Maysan Governorate, Iraq. *Annals of Tropical Medicine and Public Health*, 23, 231-238. https://doi.org/10.36295/ASRO.2020.231238

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

Ismail, N.A., Habib, S.A., Talaat, A.A., Mostafa, N.O., Elghoroury, E.A., 2019. The relation between serum hepcidin, ferritin, hepcidin: ferritin ratio, hydroxyurea and splenectomy in children with β-thalassemia. *Open Access Macedonian Journal of Medical Science*s. 7, 2434. https://doi.org/10.3889/oamjms.2019.636

Khalaf, A., Al-Saadi, H., 2022. Alpha-hemoglobin stabilizing protein gene polymorphism (rs4499252 A/G) and its association with beta-thalassemia major in Iraqi patients. *Archives of Razi Institute*. 77, 1033.

Khalaf, M.A., Al-Saadi, B.Q.H., Mohammed, H.Q., 2022. Evaluation of TLR-3, TLR4, IL-7, and IL37 immunological markers in β-thalassemia major Iraqi patients. *Iraqi Journal of Biotechnology*. 21, 115-123.

Luaibi, H.A., Mohammed, B.J., 2023. Does TNF-α 308 G/A (rs1800629) gene polymorphism associate with liver and pancreas disorders in Iraqi adults with beta thalassemia major? *Human Antibodies.* 31, 99-105. https://doi.org/10.3233/HAB-230015

Luaibi, H.A., Mohammed, B.J., 2024. Relationship of TNFα-238 G/A (rs 361525) genotypes with TNFα gene expression in liver and pancreas disorders in sample of beta thalassemia major adult Iraqi patients. *Human Antibodies.* 32, 67-74. https://doi.org/10.3233/HAB-240022

Lucarelli, G., Clift, R.A., Galimberti, M., Polchi, P., Angelucci, E., Baronciani, D., Giardini, C., Andreani, M., Manna, M., Nesci, S., 1996. Marrow transplantation for patients with thalassemia: results in class 3 patients. *Blood.* 87, 2082–2088. https://doi.org/10.1182/blood.V87.5.2082.2082

Mahmoud, H.Q., Mhana, R.S., Mohammed, A.A., 2024. Therapeutic options and management approach on thalassemia an overview. *International Journal of Medical Science and Dental Health*. 10, 17-28. https://doi.org/10.55640/ijmsdh-10-01-02

Majid, M., Mutar, M.T., Talib Hashim, H., 2021. Thalassemia awareness among Iraqi people in 2018. *Thalassemia Reports*. 10, 8655. https://doi.org/10.4081/thal.2020.8655

Meri, M.A., Al-Hakeem, A.H., Al-Abeadi, R.S., 2022. An overview on thalassemia: a review article. *Medical Science Journal for Advance Research*. 3, 26-32. https://doi.org/10.46966/msjar.v3i1.36

Mishra, A.K., Tiwari, A., 2013. Iron overload in Beta thalassaemia major and intermedia patients. *Maedica*. 8, 328.

Musallam, K.M., Taher, A.T., Rachmilewitz, E.A., 2012. β-thalassemia intermedia: a clinical perspective. *Cold Spring Harbor Perspectives in Medicine*. 2, a013482. https://doi.org/10.1101/cshperspect.a013482

Nemeth, E., 2010. Hepcidin in β-thalassemia. *Annals of the New York Academy of Sciences*, 1202, 31-35. https://doi.org/10.1111/j.1749-6632.2010.05585.x

Qurat-ul-Ain, L.A., Hassan, M., Rana, S.M., Jabeen, F., 2011. Prevalence of β-thalassemic patients associated with consanguinity and anti-HCV-antibody positivity-a cross sectional study. *Pak J Zool*. 43, 29-36.

Rivella, S., 2012. The role of ineffective erythropoiesis in non-transfusion-dependent thalassemia. *Blood Reviews*. 26, 12-15. https://doi.org/10.1016/S0268-960X(12)70005-X

Rumjon, A., 2019. Hepcidin Regulation in Chronic Kidney Disease [Thesis]. London, Inggris: King's College London. https://doi.org/10.1016/j.mpmed.2019.06.015

Shah, F., Huey, K., Deshpande, S., Turner, M., Chitnis, M., Schiller, E., Yucel, A., Moro Bueno, L., Oliva, E.N., 2022. Relationship between serum ferritin and outcomes in β-thalassemia: a systematic literature review. *Journal of Clinical Medicine*. 11, 4448. https://doi.org/10.3390/jcm11154448

Shawkat, A.J., Jwaid, A.H., 2019. Clinical complications of beta-thalassemia major. *Iraqi Journal of Pharmaceutical Sciences.* 28, 1-8. https://doi.org/10.31351/vol28iss2pp1-8

Sundaresan, D.D., Hira, J.K., Chhabra, S., Trehan, A., Khadwal, A. R., Malhotra, P., Sharma, P., Das, R., 2023. Hematological and genetic profiles of persons with co-inherited heterozygous β-thalassemia and supernumerary α-globin genes. *European Journal of Haematology*. 110, 510-517. https://doi.org/10.1111/ejh.13923

Tantiworawit, A., Kamolsripat, T., Piriyakhuntorn, P., Rattanathammethee, T., Hantrakool, S., Chai-Adisaksopha, C., Rattarittamrong, E., Norasetthada, L., Fanhchaksai, K., Charoenkwan, P., 2024. Survival and causes of death in patients with alpha and beta-thalassemia in Northern Thailand. *Annals of Medicine*. 56, 2338246. https://doi.org/10.1080/07853890.2024.2338246

Tantiworawit, A., Khemakapasiddhi, S., Rattanathammethee, T., Hantrakool, S., Chai-Adisaksopha, C., Rattarittamrong, E., Norasetthada, L., Charoenkwan, P., Srichairatanakool, S., Fanhchaksai, K., 2021. Correlation of hepcidin and serum ferritin levels in thalassemia patients at Chiang Mai University Hospital. *Bioscience Reports*. 41, BSR20203352. https://doi.org/10.1042/BSR20203352

Youssry, I., Samy, R.M., AbdelMohsen, M., Salama, N.M., 2024. The association between growth differentiation factor-15, erythroferrone, and iron status in thalassemic patients. *Pediatric Research*. 95, 1095-1100. https://doi.org/10.1038/s41390-023-02729-5

Zaman, B.A., Ibrahim, S.A., 2022. Hepcidin-to-ferritin ratio as an early diagnostic index of iron overload in β-thalassemia major patients. *Hemoglobinhemoglobin*. 46, 106-113. https://doi.org/10.1080/03630269.2022.2083969

Zarghamian, P., Azarkeivan, A., Arabkhazaeli, A., Mardani, A., Shahabi, M., 2020. Hepcidin gene polymorphisms and iron overload in β-thalassemia major patients refractory to iron chelating therapy. *BMC Medical Genetics*. 21, 1-5. https://doi.org/10.1186/s12881-020-01011-3