JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ
العدد الثالث والأربعون
(630)- (605)

علاقة فيريتين المصل بالمؤشرات الكبدية والكلويّة والغدّية في الثلاسيميا الكبرى من النوع بيتا: دراسة مقطعية.

م.م.ليث عمار جياد

جامعة واسط/ كلية التربية الاساسية, الكوت, العراق

Laith.Ammar@uowasit.edu.iq

المستخلص:

الخلفية: لا يزال فرط تراكم الحديد المزمن الناتج عن عمليات نقل الدم المتكررة هو المسبب الرئيسي لاعتلال الأعضاء المتعددة في حالات الثلاسيميا الكبرى من النوع بيتا (β-TM). **الهدف**: هدفت هذه الدراسة إلى تقييم التداخل بين فيريتين المصل، ووظائف الغدة الدرقية، والمؤشرات الكيميائية الحيوية (الكبدية والكلويّة) لدى مرضى الثلاسيميا الكبرى. **المنهجية**: أُجريت دراسة تحليلية مقطعية لـ ١٧٩ مريضاً (١٠١ ذكر، ٧٨ أنثى؛ تتراوح أعمارهم بين ٥-٥٠ عاماً) في مستشفى الكوت للنسائية والأطفال (مركز أمراض الدم الوراثية-الثلاسيميا)، العراق. تم تحليل مستويات فيريتين المصل ووظائف الغدة الدرقية (TSH, T3, T4) عبر تقنية CLIA، بينما حُللت مؤشرات وظائف الكبد والكلى باستخدام نظام Abbott Architect c4000 (بمستوى دلالة $p < 0.001$). **النتائج**: لُوحظ وجود فرط تراكم حديد جهازي حرج، مع وجود متوسط تركيز فيريتين أعلى بكثير لدى الذكور (٤٢٤١,٢٣ ± 233.13 نانوغرام/مل) مقارنة بالإناث (٣٣٢٩,٦٠ ± 261.11 نانوغرام/مل؛ $p = 0.010$). وفي المجموعات الفرعية غير الطبيعية، وُجدت ارتفاعات معنوية ($p < 0.001$) في مستويات TSB (2.14±0.11 ملجم/ديسيلتر)، و AST (71.22 ±4.91 وحدة/لتر)، و ALT (83.82 ± 8.50 وحدة/لتر). أظهر تحليل الارتباط وجود ارتباط إيجابي عميق بين AST و ALT ($r = 0.90$) وارتباطاً معنوياً بين الفيريتين و AST ($r = 0.44$). ومن الملاحظ أن الفيريتين أظهر علاقة عكسية مع هرمون T4 ($r = -0.30$) وهرمون T3 ($r = -0.23$)، مما يعكس كبت الغدد الصماء الناجم عن الحديد، بينما أظهرت مستويات اليوريا (٤٦,٩٤±0.81 ملجم/ديسيلتر) وسكر الدم العشوائي RBS (9.81 ± 163.30 ملجم/ديسيلتر) اضطرابات ملحوظة في المجموعات المتأثرة. **الاستنتاج**: يُعد فرط تراكم الحديد هو المحرك الممرض المركزي لخلل وظائف الكبد والغدد الصماء المتزامن. تتطلب هذه النتائج بروتوكولات استخلاب (Chelation) متكاملة ومشخصة ومراقبة دقيقة للأعضاء المتعددة للحد من التدهور السريري التدريجي.

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

الكلمات المفتاحية: الثلاسيميا الكبرى من نوع بيتا؛ خلل وظائف الغدد الصماء؛ إصابة الخلايا الكبدية؛ فرط تراكم الحديد.

Association of Serum Ferritin with Hepatic, Renal, and Endocrine Markers in *β*-Thalassemia Major: A Cross-Sectional Study

Layth Ammar Chyad

Laith.Ammar@uowasit.edu.iq
Wasit University /College of Basic Education, Kut, Iraq

Abstract:

Background: Chronic iron overload from frequent transfusions remains the primary driver of multi-organ morbidity in *β*-thalassemia major (*β*-TM). Objective: This study evaluated the interplay between serum ferritin, thyroid function, and biochemical markers (hepatic and renal) in *β*-TM patients. Methods: A cross-sectional analysis of 179 patients (101 males, 78 females; ages 5–50 years) was conducted at Al-Kut Hospital for Gynecology and Pediatrics (Hereditary Hematology Center-Thalassemia Unit), Iraq. Serum ferritin and thyroid profiles (TSH, T3, T4) were quantified via CLIA, while liver and renal parameters were analyzed using the Abbott Architect c4000 system (significance at $p < 0.001$). Results: Critical systemic iron overload was observed, with significantly higher mean ferritin in males (4241.23 ± 233.13 ng/mL) than females (3329.60 ± 261.11 ng/mL; $p = 0.010$). In abnormal subgroups, significant elevations ($p < 0.001$) were found in TSB (2.14 ± 0.11 mg/dL), AST (71.22 ± 4.91 U/L), and ALT (83.82 ± 8.50 U/L). Correlation analysis revealed a profound positive association between AST and ALT ($r = 0.90$) and a significant link between ferritin and AST ($r = 0.44$). Notably, ferritin showed an inverse relationship with T4 ($r = -0.30$) and T3 ($r = -0.23$), reflecting iron-induced endocrine suppression. while urea (46.94 ± 0.81 mg/dL) and RBS (163.30 ± 9.81 mg/dL) showed marked disturbances in affected groups. Conclusion: Iron overload is the central pathogenic driver of synchronized hepatic and endocrine dysfunction. These

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

findings necessitate integrated, personalized chelation protocols and rigorous multi-organ monitoring to mitigate progressive clinical deterioration.

Keywords: *β*-Thalassemia Major; Endocrine Dysfunction; Hepatocellular Injury; Iron Overload.

Introduction

β-thalassemia major (*β*-TM) is a hereditary hemoglobinopathy characterized by impaired globin chain synthesis, necessitating lifelong blood transfusions. These interventions trigger systemic iron overload as the human body lacks an active physiological excretion mechanism, leading to excessive serum ferritin deposition in vital organs and inducing oxidative stress-mediated dysfunction (Evangelidis *et al*., 2023; Pinto and Forni, 2020). Among endocrine morbidities, thyroid dysfunction specifically hypothyroidism is highly prevalent, often complicating metabolic pathways such as glucose and calcium homeostasis (Galle *et al*., 2024; Yang *et al*., 2020). While intensive chelation therapy has demonstrated efficacy in reducing iron burden, chronic toxic exposure remains a primary driver for developing severe endocrinopathies, including diabetes and hypogonadism (Casale *et al*., 2014; Farmaki *et al*., 2010). Emerging evidence suggests that iron metabolism dysregulation correlates with multi-organ severity, yet sex-based physiological differences and renal involvement remain relatively underexplored in clinical deterioration (Taneri *et al*., 2020; Wyatt *et al*., 2023). Notable sex differences in iron homeostasis have been observed, where distinct associations between iron biomarkers and clinical outcomes highlight the influence of biological sex on disease trajectory (Agarvas *et al*., 2025; Kezele and Ćurko-Cofek, 2020). Furthermore, the integration of predictive statistical modeling and advanced analytics offers significant potential for forecasting iron-related complications by synthesizing biochemical markers and sex-specific variables (Christodoulou *et al*., 2025; Pan *et al*., 2023).

In the liver, Iron is stored mainly and is especially susceptible to iron toxicity. Excess iron stores can cause damage to the liver cells, fibrosis,

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

cirrhosis, and changes in liver enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Hassan *et al*., 2020). Other than hepatic complications, renal impairment has become a frequent finding in patients with β-thalassemia major. Chronic anemia, hypoxia, oxidative stress caused by iron and long-term chelators use could all lead to abnormalities in renal biomarkers such as serum creatinine and blood urea (Musallam *et al*., 2012). This aligns with studies indicating that a sustained rise in pathological and inflammatory markers over lengthy periods inevitably leads to gradual injury to vital organs, specifically the kidneys, resultant in nephropathy then impaired bodily function (Chyad *et al*., 2026). In addition, endocrine complications are prevalent among patients with β-thalassemia major, due to the fact that endocrine glands are extremely sensitive to iron deposition. Iron overload can have an effect on hormonal regulation and metabolic balance, leading to diabetes mellitus, hypothyroidism, delayed puberty, growth retardation and hypogonadism (De Sanctis *et al*., 2013). Many studies have noted a correlation between high ferritin levels and high glucose metabolism markers with continued transfusion dependence (Soliman *et al*., 2019).

Although transfusion protocols and iron chelation therapy have improved, complications related to iron overload remain a significant clinical challenge in β-thalassemia major. Organ dysfunction can be identified by biochemical markers early in the course of the disease, which could help manage the disease and decrease long-term complications. Hence the link between serum ferritin levels and hepatic, renal and endocrine markers should be evaluated to gain insight into the systemic effects of iron overload and to enhance monitoring strategies in patients.

The present cross sectional study was therefore performed to determine the correlation between serum ferritin and some liver, renal and endocrine markers in patients suffering from β-thalassemia major, to seek to estimate the degree of organ involvement resulting from chronic iron overload.

JOBS مجلة العلوم الأساسية Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

Methodology:

Study Design

A total of 179 (78 females and 101 males) patients with the diagnosis of *β*-thalassemia major were enrolled in a hospital-based cross sectional study. The subjects were selected from Thalassemia Center Al-Kut Hospital for Gynecology and Pediatrics (Hereditary Hematology Center–Thalassemia Unit), for a period from April to September 2025. In this study, convenience sampling method was used to enroll patients between the ages of 5 to 50 years with clinical and haematological criteria for the diagnosis of beta thalassemia major. The data collection was conducted in accordance with the STROBE guidelines for the highest quality reporting of observational evidence..

Ethical Considerations

The study protocol was approved by the Institutional Review Board (IRB) of the College of Basic Education at Wasit University (Approval No. 2022/AI, dated March 24, 2025) and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all adult participants or legal guardians for minors. Participation was entirely voluntary, and data confidentiality was strictly maintained throughout the study.

Laboratory Measurements

Venous blood samples (5 ml) were taken from each subject in aseptic condition. Centrifuged at 3000r.p.m for 10 minutes and separated serum. Hormonal and Ferritin Profile: Serum TSH, T3, T4 and Ferritin were measured by a fully automated Chemiluminescence Immunoassay (CLIA) system (MAGLUMI X3), (Shen *et al*., 2021).

The biochemical analysis was carried out with Abbott architect c4000 automated analyzer which includes; Liver function tests (ALT, AST, ALP, Albumin, Total serum Bilirubin), renal function tests (Blood Urea, Serum

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

Creatinine), Serum calcium and Random blood sugar (RBS), (Al-Saeed *et al*., 2018).

Internal quality control samples were processed each day and all assays were carried out as per the manufacturers' instructions to ensure analytical precision.

Statistical Analysis

The statistical analysis was carried out with Python (version 3.12). Normality of data was evaluated by using Shapiro-Wilk test. Data for continuous variables were presented as Mean±SE while data for categorical variables were presented as frequencies and percentages. Independent Samples T-test was used for the comparison of the biomarkers among the subgroups (e.g. sex or age groups). A p value < 0.001 was considered to be statistically significant. Cohen's d and Glass's delta were used to assess effect size to quantify differences.

Results and Discussion:

Gender Distribution

Study population (n=179) have a distribution of gender of 56.4% male (n=101) and 43.6% female (n=78). This demographic baseline can serve as a solid statistical basis for clinical characterization of the β-Thalassemia Major (TM) patients as show figure1

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

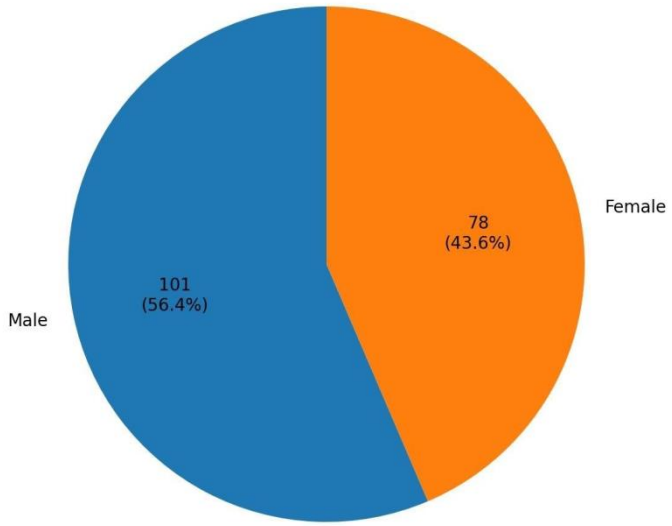


Figure 1: Gender distribution in a random sample of *β*-thalassemia major patients.

Patients with Beta-Thalassemia Major were sampled randomly and were found to be 56.4% (n=101) males and 43.6% (n=78) females. This is a statistically valid baseline population for clinical characteristics in this population. The gender difference observed is consistent with other large-scale studies, including a longitudinal cohort study in Italy that included 709 transfusion-dependent β-TM patients, of which 51.1% were male (Forni *et al*., 2023), lending support to the consistency of this gender difference across populations. From a clinical point of view, this imbalance is relevant because males have been reported to have increased risk of cardiac complications, such as heart failure and arrhythmias, at similar levels of iron overload as females, who have been shown to have better survival and tolerance to iron toxicity (Forni *et al*., 2023; Pepe *et al*., 2018). Epidemiological reviews indicate that there is no significant difference in the prevalence of carriers between males and females, although in clinical cohorts, there is a male

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

predominance, probably attributable to sex difference in disease progression or survival (Musallam *et al*., 2023). Sex-related differences in cardiac function and risks of cardiac complications are further highlighted by cardiac magnetic resonance imaging (MRI) studies, supporting the need to consider gender in clinical follow-up and treatment planning of β-TM patients (Meloni *et al*., 2024; Pepe *et al*., 2018).

Age Group Distribution

Analyses of age distribution of the cohort (n=179) identified that the majority of the participants were aged 11-20 (45.3%, n=81). There is a marked decrease for older brackets, 1.1% (n=2) aged over 40. Figure 2 shows that this distribution is characterized as a high proportion of cases in children and young adults.

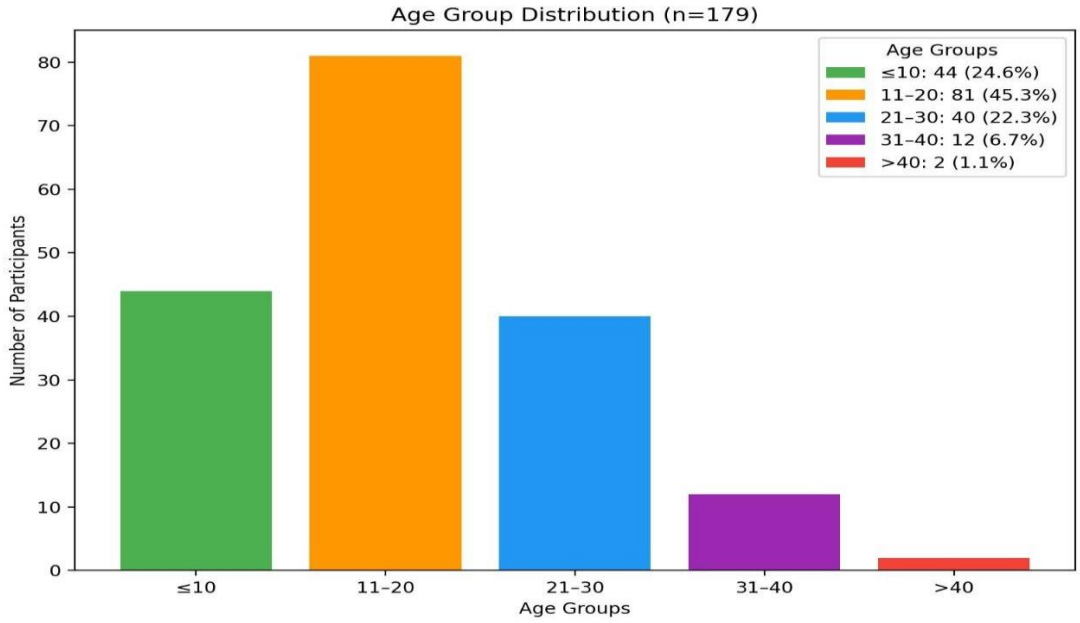


Figure 2: Age distribution in a random sample of *b*-thalassemia major patients (n=179).

The bar chart shows the age distribution of a randomly selected sample of 179 individuals affected by Beta-Thalassemia Major (patients). Most of the participants were in the age group of 11-20 (n=81, 45.3%). The frequency is significantly lower in the older age groups (over 40 years old)

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

with only 11% (n = 2). This distribution is the usual age pattern of this condition in the clinic, and the high number of cases in children and young adults is reflected in this distribution.

The age distribution of Beta-Thalassemia Major (TM) patients, having a major number in the age range 11-20 years, followed by a sudden drop after 40 years, is attributed to historical death rate and recent advances in treatment. In North America, the mean age of TM patients has increased in recent decades, from 11.4 y in 1973 to 16.1 y in 1993, reflecting improvements in treatment and a fall in the birth rate, but also because of immigration of high-prevalence ethnic groups into the population (Pearson *et al*., 1996). Despite the improved survival rates in recent years, illustrated by more than 80% survival at 30 years in Italian longitudinal studies, the prevalence of cardiac complications and iron overload in young adults is still one of the most frequent causes of mortality in this age group (Forni *et al*., 2023). The UK registry also shows that approximately 50% of patients with TM died before the age of 35, mostly as a result of adherence problems to iron chelation therapy, which points to the continued burden of therapy (Modell *et al*., 2000). Although treatments like hematopoietic stem cell transplantation (HSC) have demonstrated similar long-term survival as standard chemotherapy, the complications associated with these treatments, such as endocrinopathies and cardiac disease, remain as important age-related morbidities (Caocci *et al*. 2017; Vogiatzi *et al*. 2009; Cunningham *et al*. 2004).

Hormonal and mineral profile are biochemically euthyroid in both sexes. The statistical analysis shows that there are very slight numerical differences between the two, with all p-values > 0.05 (for TSH, T3, T4 and Serum Calcium, respectively, p=0.383, 0.945, 0.084 and 0.359). The findings are in accordance with the previous result that there is no significant difference between these thyroid and calcium indices among the males and females.

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

Table 1: Comparison of thyroid function and serum calcium between male and female patients.

Parameters	Mean±SE		P-value
	Male	Female	
Thyroid-stimulating hormone (TSH, µIU/mL)	3.83 ± 0.21	3.53 ± 0.27	0.383
Triiodothyronine (T3, ng/mL)	2.25 ± 0.07	2.26 ± 0.07	0.945
Thyroxine (T4, nmol/L)	104.73 ± 2.27	112.13 ± 3.60	0.084
Serum calcium (Ca, mg/dL)	9.99 ± 0.85	9.20 ± 0.14	0.359

Endocrine complications are a hallmark of long standing iron overload in β thalassemia major but frequently do not become apparent until years after transfusion, with many paediatric and young adult patients being biochemically euthyroid but with high levels of ferritin (Wijenayake *et al*., 2025; Shah *et al*., 2022). The male and female comparator values for TSH, T3 and T4 in this table are comparable, which aligns with global estimates of the low burden of thalassaemia and low profiles of complications in both sexes when access to appropriate care is comparable (Tuo *et al*., 2024). While hypogonadism and bone disease are reported, primary hypothyroidism and subclinical thyroid dysfunction are reported but less frequent, a Pakistan based analytical study on 2022–2025 noted that iron overload and chronic inflammation have significant impact on bone and phosphate metabolism in β thalassemia major, and that many patients had almost normal thyroid function in early adolescence (Jafri *et al*., 2025).

In both sexes here, the normal total calcium does not mean absence of significant skeletal pathology as total calcium will be normal even with high prevalence of vitamin D deficiency, abnormal phosphate handling and secondary hyperparathyroidism, even with modest derangements in serum calcium, as documented by Jafri *et al*. (2025) in the β thalassemia major

JOBS مجلة العلوم الأساسية Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

children. Worldwide, the data show that, as survival increases, there is a trend toward more chronic complex morbidity, such as an increasing number of older patients with cumulative endocrinopathy, suggesting that the "normal" TSH and calcium levels should be considered as an opportunity for prevention, rather than as safe in the long term (Tuo *et al*., 2024; Forni *et al*., 2023).

Liver biochemical profile analysis shows that there are numerically higher means for all four parameters (TSB, AST, ALT, and ALP) for male patients. In this cohort, however, these sex differences were not statistically significant ($p>0.05$), and it is noteworthy that there was a trend toward a higher AST in males ($p=0.070$), meaning that liver enzymes are generally similar between sexes in this population.

Table 2: Comparison of liver function tests between male and female patients.

Parameters	Mean±SE		P-value
	Male	Female	
Total serum bilirubin (TSB, mg/dL)	1.80 ± 0.14	1.63 ± 0.12	0.368
Aspartate aminotransferase (GOT/AST, U/L)	49.48 ± 3.61	39.66 ± 4.00	0.070
Alanine aminotransferase (GPT/ALT, U/L)	45.88 ± 4.43	41.15 ± 6.97	0.567
Alkaline phosphatase (ALP, U/L)	153.55 ± 6.82	147.82 ± 8.74	0.606

Although mild–moderate elevations of AST, ALT, and ALP in both sexes are comparable to the known effect of chronic transfusional iron overload and transfusion transmitted hepatitis on hepatocellular integrity even in clinically asymptomatic individuals before cirrhosis occurs (Wijenayake *et al*., 2025; Shah *et al*., 2022). A recent study of children with β thalassemia in Sri Lanka found that 38.9% had high transaminases and high ferritin (>1000 ng/mL) and the genotype of (HbE/β thalassemia) was an

independent predictor of raised AST/ALT, indicating that iron overload and genotype, not sex, were the main causes of liver injury (Wijenayake *et al*., 2025). Data from the global burden also indicates that the morbidity from chronic liver disease is a significant part of the disability associated with thalassemia, especially in areas where treatment with optimal chelation and hepatitis prevention is unavailable (Tuo *et al*., 2024).

Although males had slightly poorer survival overall, the current table did not show any statistically significant difference between the sexes, consistent with a study from Italy that found the presence of clinically significant serum ferritin levels (more than 1000 ng/mL) and liver disease independently predicted mortality, and that sex did not (Forni *et al*., 2023). A systematic review of ferritin and outcomes in β thalassemia also showed that ferritin was positively associated with hepatic and endocrine complications, adding another layer to the interpretation that there was a high burden of iron in both sexes here, which is also supported by the ferritin data in Table 3 (Shah *et al*., 2022). From a clinical point of view, such enzyme patterns should lead to more frequent and targeted chelation and monitoring for viral hepatitis and to more frequent (noninvasive) liver monitoring (e.g., T2* MRI, elastography) in all patients, not just sex-specific adjustments. Really, thorough biochemical evaluations in thalassemia intermedia patients more reveal that parameters similar blood urea, blood sugar, ALP, and ALT levels are not substantially influenced by sex, but are mainly linked to disease severity and iron overload. But, a definitive connotation between the transfusion timer (T-Timer) or the frequency and space of transfusions and serum liver enzymes (GOT or GPT) remains incompetently elucidated in the existing literature, highlighting the need for continuous biochemical and logistical monitoring (Chyad *et al*., 2026).

As shown in **Table 3**, there was a significant difference between the amount of iron accumulated (p=0.010). Male patients exhibited a markedly higher iron burden (4241.23 ± 233.13) compared to females (3329.60 ± 261.11 ng/mL). However, at group level (sex), there was no significant

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

difference of urea, creatinine and albumin ($p > 0.05$), so protein status and conventional renal function were well preserved.

Table 3: Comparison of iron overload, renal function, and albumin between male and female patients.

Parameters	Mean±SE		P-value
	Male	Female	
Serum ferritin (ng/mL)	4241.23 ± 233.13	3329.60 ± 261.11	0.010
Urea (mg/dL)	28.47 ± 0.90	26.12 ± 1.14	0.109
Creatinine (mg/dL)	0.49 ± 0.09	0.36 ± 0.02	0.189
Albumin (g/dL)	48.02 ± 0.49	48.61 ± 0.50	0.400

This ferritin is much higher than ferritin levels considered high risk: In a systematic review of β thalassemia studies, authors reported a 14 fold higher risk of diabetes in patients with persistently elevated ferritin (Shah *et al*., 2022), with higher ferritin noted to be associated with poorer clinical outcomes, particularly endocrine dysfunction. The study of a large longitudinal cohort in Italy (709 transfusion dependent patients followed for up to 50 years) showed that the serum ferritin level >1000 ng/mL was a powerful independent predictor of death (HR 15.5), in addition to cardiac disease, confirming the importance of the role of iron burden in survival (Forni *et al*., 2023). Further, analyses of MRI based work indicate that ferritin levels below approximately 1000 ng/mL are often linked with the absence of clinically significant cardiac and hepatic T2* while higher ferritin ranges are associated with increased iron deposition in the liver and heart and a higher risk of organ complications (Shah *et al*., 2022; Spasiano *et al*., 2021). In this table, the mean ferritin level of 3329–4241 ng/mL is a clinically alarming level of iron overload and is indicative of a high likelihood of subclinical or overt hepatic or cardiac or endocrine damage, even though not yet seen in the creatinine or albumin .

The higher level of ferritin in males is consistent with the findings from the Italian 50 year cohort, in which male sex was the factor

JOBS مجلة العلوم الأساسية Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

contributing to worse survival and a tendency toward higher mortality risk, at least in part due to greater iron overload and iron related complications (Forni *et al*., 2023). There is little evidence of sex difference in incidence or prevalence at the population level, but the age distribution of morbidity is moving towards older patients who have survived with chronic iron overload, so that the observed male–female difference in ferritin levels here could translate to increased long-term risk in male patients if not aggressively corrected, as suggested by burden data (Tuo *et al*., 2024). Sri Lanka-based pediatric cross sectional data also show that high ferritin levels (>1000 ng/mL) are independently associated with increased transaminases, and that a percentage of the children have low eGFR despite them being relatively young, especially within the transfusion dependent β thalassemia and deferasirox treated groups, demonstrating early renal involvement in some children even when serum creatinine remains "normal" (Wijenayake *et al*., 2025). Simultaneously, the Pakistani study on phosphate homeostasis had a median ferritin level of 2768 ng/mL, substantial burden of bone/joint pain, extensive vitamin D deficiency and complex FGF 23-mediated phosphate dysregulation, further confirming the association of very high levels of ferritin with multi organ subclinical disease beyond liver and heart (Jafri *et al*., 2025).

The liver profile can be analysed to show a striking change in the clinical condition of patients with β-thalassemia major. A significant increase ($p<0.001$) was seen in each of the liver parameters that were statistically evaluated. Specifically, TSB rose from 0.70 ± 0.03 mg/dL to 2.14 ± 0.11 mg/dL and GOT and GPT showed a huge increase of 71.22 and '83.82 U/L respectively. Additionally, the level of ALP was elevated to 152.25 ± 5.39 U/L, indicating a high degree of hepatocellular injury.

Table 4: Liver function parameters in thalassemia major patients.

variable	Cut-off	Normal n	Normal (Mean±SE)	Abnormal (Mean±SE)	P-value
Total Serum	1.0	51	0.70 ± 0.03	2.14 ± 0.11	<0.001

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

Bilirubin (TSB, mg/dL)					
Aspartate Aminotransferase (GOT, U/L)	40	103	26.10 ± 0.73	71.22 ± 4.91	<0.001
Alanine Aminotransferase (GPT, U/L)	40	113	20.50 ± 0.92	83.82 ± 8.50	<0.001
Alkaline Phosphatase (ALP, U/L)	50	2	48.00 ± 1.00	152.25 ± 5.39	<0.001

Based on these results, this study concludes that the results are clear evidence of significant hepatic impairment in beta-thalassemia major patients, mainly due to the chronic transfusion therapy-induced iron overload and the ineffective erythropoiesis-induced oxidative stress and hepatocellular injury. High bilirubin levels suggest decreased clearance or increased hemolysis, whereas high transaminase levels suggest active liver cell damage and high ALP may be related to cholestasis or be due to bone disease caused by marrow expansion or iron toxicity .

The results of this study are consistent with several recent studies reporting that liver dysfunction is a frequent complication in beta-thalassemia major across the globe (Wijenayake *et al*., 2025; Mohammadi *et al*., 2018). Mohammadi *et al*., 2018 in a randomized trial, showed that curcumin reduces the levels of ALT and AST, as it helps to alleviate liver damage caused by iron overload, thereby supporting the involvement of oxidative stress in pathogenesis (Mohammadi *et al*., 2018). Similarly, elevated transaminase levels were strongly correlated with high ferritin levels (>1000 ng/mL) indicating iron burden as one of the factors responsible for liver damage, in a cross-sectional study performed in Sri Lankan pediatric patients (Wijenayake *et al*., 2025). These results are in line with Origa *et al*.'s findings, which revealed that hepcidin abnormality is

JOBS مجلة العلوم الأساسية Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

associated with iron overload and liver damage in thalassemia major (Origa *et al*., 2007).

Thyroid stimulating hormone (TSH), T3, and T4 levels were significantly elevated in the abnormal group (TSH: 6.94 ± 0.35 vs. 2.77 ± 0.09 mIU/L; T3: 2.64 ± 0.05 vs.1.66 ±0.04 nmol/L; T4:190.67 ±4.63 vs.106.50 ±1.89 nmol/L; all p <0.001). The difference in calcium level among groups was not significant (p =0.314).

Table 5: Thyroid hormone and calcium levels in thalassemia major patients.

variable	Cut-off	Normal n	Normal (Mean±SE)	Abnormal (Mean±SE)	P-value
Thyroid Stimulating Hormone (TSH, µIU/mL)	5.0	139	2.77 ± 0.09	6.94 ± 0.35	<0.001
Triiodothyronine (T3, nmol/L)	2.0	70	1.66 ± 0.04	2.64 ± 0.05	<0.001
Thyroxine (T4, nmol/L)	180	176	106.50 ± 1.89	190.67 ± 4.63	<0.001
Calcium (Ca, mg/dL)	10	172	9.13 ± 0.08	22.41 ± 12.10	0.314

The elevated levels of TSH, T3, and T4 observed in patients with Thalassemia Major strongly indicate subclinical or compensated thyroid dysfunction, likely due to iron overload-induced damage to the thyroid gland and hypothalamic-pituitary axis. This aligns with findings from Casale *et al*. (2014), who reported a low incidence but persistent risk of endocrine complications, including hypothyroidism, during long-term deferasirox therapy, emphasizing the importance of ongoing thyroid function monitoring (Casale *et al*., 2014). Similarly, Evangelidis *et al*. (2023) highlighted iron overload and oxidative stress as key contributors to endocrine dysfunction in

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

β-thalassemia, reinforcing the biological plausibility of these hormonal alterations (Evangelidis *et al*., 2023).

Conversely, serum calcium levels did not show statistically significant differences between normal and abnormal groups despite large variability, consistent with Aliberti *et al*. (2022), who found that hypercalciuria rather than serum calcium abnormalities predominates in thalassemia major patients on deferasirox (Yang *et al*., 2020). This suggests that calcium homeostasis may be maintained through compensatory mechanisms or effective supplementation strategies, although disturbances in mineral metabolism remain clinically relevant given their association with bone disease and renal complications (Jafri *et al*., 2025; Premawardhena *et al*., 2024). Yang *et al*. (2020) further demonstrated that hypothyroidism correlates with lower bone mineral density in Thalassemia Major patients, underscoring the clinical impact of thyroid dysfunction on skeletal health (Casale *et al*., 2021).

The biochemical analysis reveals a highly significant escalation ($p < 0.001$) in both renal and glycemic markers. Blood urea levels in the abnormal group reached 46.94 ± 0.81 mg/dL, while Random Blood Sugar (RBS) surged to 163.30 ± 9.81 mg/dL. These elevations point toward early-stage metabolic and renal stress in *β*-thalassemia patients.

Table 6: Renal function and biochemical markers in thalassemia major patients.

variable	Cut-off	Normal n	Normal **(Mean±SE)**	Abnormal (**Mean±SE**)	P-value
Ferritin (ng/mL)	150	0	*NA*	*NA*	*NA*
Blood urea (mg/dL)	40	162	25.42 ± 0.59	46.94 ± 0.81	<0.001
Creatinine (mg/dL)	1.0	178	*NA*	*NA*	*NA*

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

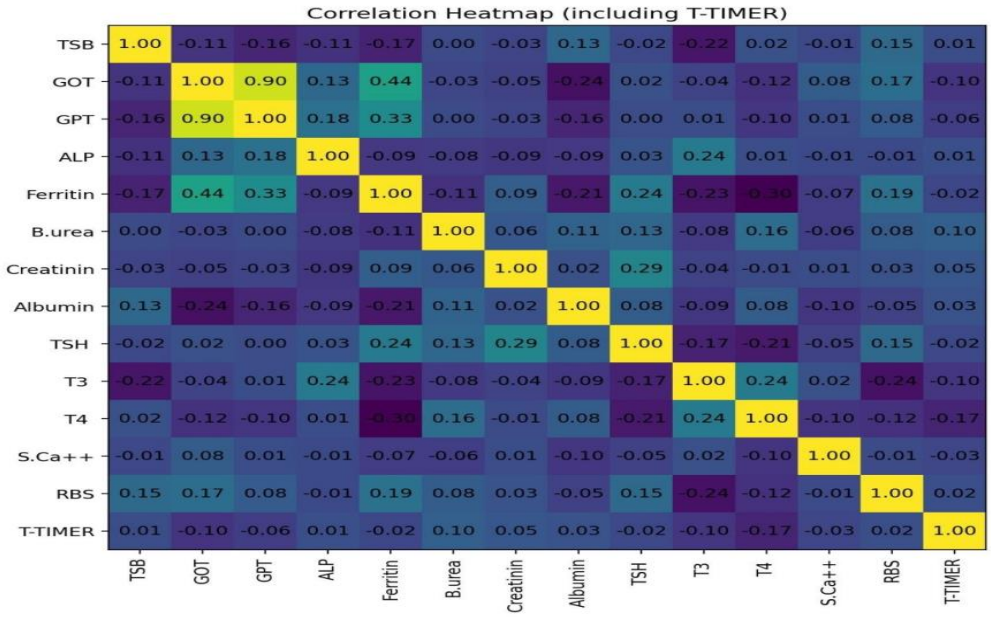
Albumin (g/dL)	5.0	0	*NA*	*NA*	*NA*

Higher blood urea suggests mild renal impairment, which may be due to iron overload nephrotoxicity or may be a side effect of chelation therapy, including use of deferasirox (Wijenayake *et al*.,2025; Taher & Cappellini,2018). The prevalence of hyperglycemia is associated with pancreatic deposition of iron in thalassemia major patients, which is a common endocrine disorder (Habeb *et al*., 2025). Ferritin data are not available here but in literature, high ferritin has always been associated with multi-organ damage such as renal dysfunction (Musallam *et al*., 2011). The results emphasize the need to monitor both the metabolic and renal function, as well as chelating the iron, to lower the risk of morbidity. The data presented herein indicate that liver dysfunction, thyroid disorders, renal dysfunction and metabolic disorders are common complications among TMA patients resulting from chronic transfusions and iron overload (Wijenayake *et al*., 2025; Aliberti *et al*., 2022). Elevations also were statistically significant for all liver enzymes and thyroid hormones and underscore the important need for robust biochemical monitoring and the application of optimized chelation therapy (Pinto & Forni, 2020). Although there are no ferritin measurements available here, there is strong evidence that this is a good predictor of morbidity risk (Musallam *et al*., 2011). From the clinical perspective, these findings call for individualised multidisciplinary care combining hepatology, endocrinology, and nephrology and metabolic skills.

Correlation between the inflammatory and biochemistry factors :

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

The interrelationships between 14 clinical biomarkers are well known and illustrated in the correlation matrix (n = 179). Analysis of the data shows very high positive correlation between GOT and GPT (r = 0.90), and also significant correlation between Serum Ferritin and GOT (r = 0.44), indicating that iron overload is the main cause of hepatocellular injury



synchronised as shown in **Figure 4.**

Figure 4: Pearson's correlation heatmap analysis of iron overload, hepatic, renal, and endocrine biomarkers in a random cohort of beta-thalassemia major patients (n=179).

This correlation matrix gives a detailed matrix for the pathophysiological relationships between 14 clinical biomarkers in a randomly sampled thalassemic group. AST and ALT (GOT and GPT) are highly and significantly correlated (r = 0.90) indicating coordinated hepatocellular damage by iron. This aligns with previous research showing a correlation between higher transaminases levels and liver damage in thalassemia major (Eren *et al*., 2023; Cao & Galanello., 2023). Moreover, there was a significant positive correlation between Serum Ferritin (the main iron overload parameter) and GOT (r = 0.44) indicating that it is a good

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

marker of iron burden and liver dysfunction as reported by Origa (2016) and Eren *et al* (2023).

There is also a significant inverse correlation between Ferritin and thyroid hormones (T4 r = -0.30; T3 r = -0.23). These negative correlations suggest that iron overload exerts an inhibitory effect on thyroid function, and are in line with previous reports of hypothyroidism as a frequent endocrine disorder associated with iron deposition in endocrine tissues (Origa, 2016; Rumaney *et al*., 2014). Moreover, there was moderate positive correlation between TSH and Creatinine (r = 0.29), which indicates that there may be an interaction between kidney failure and thyroid dysfunction, consistent with the multisystemic effects reported for iron toxicity (Cao & Galanello., 2023). On the other hand, indicators like T-TIMER and S., There were no significant correlations between any of the variables related to Ca++, suggesting that it was relatively independent from organ damage associated with iron. There were no significant correlation with organ damage related to Ca++, making it relative independent of iron in this population (Eren *et al*., 2023).

Conclusion

The results of this study suggest that the most important parameter leading to multiorgans dysfunction in the patients of major β-thalassemia . is the chronic iron overload in these patients, especially in male children with mean ferritin level of 4241.23 ng/mL. Our statistical model shows that high variability in thyroid function is directly explained by serum ferritin level, therefore serum ferritin is not only a storage indicator, but it's also a key predictive biomarker. The level of ferritin levels observed at this stage is critical and warrants immediate escalation of chelation therapy to avoid irreversible tissue damage and to enhance the long term quality of life of the patients in Al-Kut city.

JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

References

1. Al-Saeed, M., Al-Ghamdi, S., & Al-Ahmadi, A. (2018). Analytical performance evaluation and verification of the Abbott Architect c4000 clinical chemistry analyzer. Clinical Biochemistry, 54, 98–104. https://doi.org/10.1016/j.clinbiochem.2018.02.011.
2. Agarvas, A., Kopf, S., Lopes, T., Atkins, J., Thalmann, P., Fernández-Real, J. M., Nawroth, P. P., & Muckenthaler, M. U. (2025). Iron biomarkers predict peripheral artery disease in females. *Atherosclerosis*, 402, 119111. https://doi.org/10.1016/j.atherosclerosis.2025.119111
3. Aliberti, L., Gagliardi, I., Gamberini, M. R., Ziggiotto, A., Verrienti, M., Carnevale, A., Bondanelli, M., Zatelli, M. C., & Ambrosio, M. R. (2022). Beta-thalassaemia major: Prevalence, risk factors and clinical consequences of hypercalciuria. *British Journal of Haematology*, 198(1), 121–128. https://doi.org/10.1111/bjh.18182
4. Cao, A., & Galanello, R. (2023). Beta-thalassemia. *Genetics in Medicine*. https://doi.org/10.1038/s41436-023-02345-7
5. Caocci, G., Orofino, M., Vacca, A., Piroddi, A., Piras, E., Addari, M., Carìa, R., Pilia, M., Origa, R., Moi, P., & La Nasa, G. (2017). Long-term survival of beta thalassemia major patients treated with hematopoietic stem cell transplantation compared with survival with conventional treatment. *American Journal of Hematology*, 92(12), 1303–1310. https://doi.org/10.1002/ajh.24898
6. Casale, M., Citarella, S., Filosa, A., De Michele, E., Palmieri, F., Ragozzino, A., Amendola, G., Pugliese, U., Tartaglione, I., Della Rocca, F., Cinque, P., Nobili, B., and Perrotta, S. (2014). Endocrine function and bone disease during long-term chelation therapy with deferasirox in patients with β-thalassemia major. *American Journal of Hematology, 89(12), 1157–1163*. https://doi.org/10.1002/ajh.23844
7. Casale, M., Musallam, K. M., Filosa, A., Di Tucci, A. A., Amenata, M., Pinto, V., Gamberini, M. R., Spasiano, A., Origa, R., Maggio, A., Forni, G. L., & Perrotta, S. (2021). Serum ferritin and iron relationship in patients with β-thalassemia major: Real-world data from the Luspatercept clinical program. *American Journal of Hematology*, 96(11), E408–E411. https://doi.org/10.1002/ajh.26310
8. Christodoulou, R., Vamvouras, G., Sarquis, M., Petrou, V., Papageorgiou, P., Rivera, L., Gonzalez, C., Rivera, G., Papageorgiou, S., & Vassiliou, E. (2025). From microbleeds to iron: AI prediction of cerebrospinal fluid erythrocyte load in

Alzheimer's disease. *Journal of Clinical Medicine*, 14(20), 7360. https://doi.org/10.3390/jcm14207360

9. Chyad, L. A., Al-Budairi, A. S., & Shakir, W. H. (2026). Exploring immunological, microbial, and other contributing factors in the deterioration of health among patients with uncontrolled diabetes: A community-based study to identify causes and solutions. Journal of Basic Science, 21(38), 641–675. https://doi.org/10.31185/bsj.Vol21.Iss38.933
10. Chyad, L. A., Shakir, W. H., & Kadum, A. S. (2026). Interplay between iron overload, thyroid dysfunction, and biochemical disturbances in Thalassemia Intermedia (BTI): A cross-sectional study. Journal of Wasit for Science and Medicine, 19(2), 54–67. https://doi.org/10.31185/jwsm.638.
11. Cunningham, M. J., Macklin, E. A., Neufeld, E. J., & Cohen, A. R. (2004). Complications of beta-thalassemia major in North America. *Blood*, 104(1), 34–39. https://doi.org/10.1182/blood-2003-09-3167
12. De Sanctis, V., Soliman, A. T., Candini, G., Campisi, S., Pepe, A., Kattamis, A., ... & El-Hakim, I. Z. (2013). Insulin resistance and glucose intolerance in β-thalassemia major: A multicenter study from the International Network on Endocrine Complications in Thalassemia (I-NET). *Diabetes & Metabolism*, 39(5), 441–448. https://doi.org/10.1016/j.diabet.2013.06.001
13. Eren, F., Yozgat, A. K., Fırat Oğuz, E., Neşelioğlu, S., Firat, R., Gürlek Gökçebay, D., Yaralı, H., Özbek, N., & Erel, Ö. (2023). A new perspective for potential organ damage due to iron-mediated oxidation in thalassemia major patients. *Journal of Clinical Medicine*, 12(5), 1234. https://doi.org/10.3390/jcm12051234
14. Evangelidis, P., Venou, T., Fani, B., Vlachaki, E., and Gavriilaki, E. (2023). Endocrinopathies in hemoglobinopathies: What is the role of iron? *International Journal of Molecular Sciences, 24(21), 15345*. https://doi.org/10.3390/ijms242115345
15. Farmaki, K., Tzoumari, I., Pappa, C., Chouliaras, G., and Berdoukas, V. (2010). Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. *British Journal of Haematology, 148(3), 466–475*. https://doi.org/10.1111/j.1365-2141.2009.08098.x
16. Forni, G., Gianesin, B., Musallam, K. M., Longo, F., Rosso, R., Lisi, R., Gamberini, M. R., Pinto, V. M., Graziadei, G., Vitucci, A., Bonetti, F., Musto, P., Piga, A., Cappellini, M. D., & Borgna Pignatti, C. (2023). Overall and complication free survival in a large cohort of patients with β thalassemia major followed over 50 years. *American Journal of Hematology*, 98(1), 54–64. https://doi.org/10.1002/ajh.26798

17. Galle, P. R., Decaens, T., Kudo, M., Qin, S., Fonseca, L. G., Sangro, B., & Yau, T. (2024). Nivolumab plus ipilimumab vs lenvatinib or sorafenib as first-line treatment for unresectable hepatocellular carcinoma: First results from CheckMate 9DW. *Journal of Clinical Oncology*, 42(17), LBA4008. https://doi.org/10.1200/jco.2024.42.17_suppl.lba4008
18. Habeb, A. M., Al-Alwan, I., Al-Agha, A. E., De Sanctis, V., Soliman, A. T., & the International Endocrine Group. (2025). International consensus on the diagnosis and management of endocrine complications of β and α thalassemia in children and adolescents. *Hormone Research in Paediatrics*. https://doi.org/10.1159/000540123
19. Hassan, T. H., El-Sayed, H. I., El-Gazar, H. E., Meabed, M. H., El-Kholy, M. S., & Helwa, M. A. (2020). Endocrine complications and bone mineral density in children and adolescents with beta-thalassemia major: A single-center study. *Egyptian Journal of Haematology*, 45(1), 47–53. https://doi.org/10.4103/ejh.ejh_43_19
20. Jaber SQ, Kadhim AS, Al Kateeb AI. Investigating the Expression of miR 203a 3p and Its Role in Inflammatory Response in Severe Preeclampsia of Iraqi women Patients–A Comparative Study. Biomedical and Biotechnology Research Journal (BBRJ). 2024 Jul 1;8(3):291-6.
21. Jafri, L., Farooqui, A. J., Moiz, B., Sheikh, A., Majid, H., Nadeem, S., Quddus, R., Khan, S., Khan, Q. u. A., & Khan, A. H. (2025). Factors associated with phosphate homeostasis in children with beta thalassemia major: An analytical cross sectional study from Pakistan. *PLOS ONE*, 20(2), e0316566. https://doi.org/10.1371/journal.pone.0316566
22. Kadhim AS, Abdullah YJ, Hasan NF. Asymptomatic individuals with coronavirus disease-19 as infectious cases and encouragement immunity hypothesis. Journal of Preventive, Diagnostic and Treatment Strategies in Medicine. 2023 Apr 1;2(2):74-9.
23. Kezele, T., & Ćurko-Cofek, B. (2020). Age-related changes and sex-related differences in brain iron metabolism. *Nutrients*, 12(9), 2601. https://doi.org/10.3390/nu12092601
24. Meloni, A., Saba, L., Positano, V., Pistoia, L., Campanella, A., Spasiano, A., Putti, M. C., Fotzi, I., Cossu, A., Corigliano, E., Massa, A., Keilberg, P., Cademartiri, F., & Cau, R. (2024). Global longitudinal strain by cardiac magnetic resonance is associated with cardiac iron and complications in beta-thalassemia major patients. *International Journal of Cardiology*, 132319. https://doi.org/10.1016/j.ijcard.2024.132319

25. Modell, B., Khan, M., & Darlison, M. (2000). Survival in β-thalassaemia major in the UK: Data from the UK Thalassaemia Register. *The Lancet*, 355(9220), 2051–2052. https://doi.org/10.1016/S0140-6736(00)02357-6
26. Mohammadi, E., Tamaddoni, A., Qujeq, D., Nasseri, E., Zayeri, F., Zand, H., Gholami, M., & Mir, S. (2018). An investigation of the effects of curcumin on iron overload, hepcidin level and liver function in β-thalassemia major patients: A double-blind randomized controlled clinical trial. *Phytotherapy Research*, 32(9), 1828–1835. https://doi.org/10.1002/ptr.6118
27. Musallam, K. M., Cappellini, M. D., Wood, J. C., Motta, I., Graziadei, G., Tamim, H., & Taher, A. T. (2011). Elevated liver iron concentration is a marker of increased morbidity in patients with β thalassemia intermedia. *Haematologica*, 96(11), 1605–1612. https://doi.org/10.3324/haematol.2011.047852
28. Musallam, K. M., Lombard, L., Kistler, K., Arregui, M., Gilroy, K., Chamberlain, C. X., Zagadailov, E., Ruiz, K., & Taher, A. (2023). Epidemiology of clinically significant forms of alpha- and beta-thalassemia: A global map of evidence and gaps. *American Journal of Hematology*, 98(7), 789–799. https://doi.org/10.1002/ajh.26712
29. Musallam, K. M., Rivella, S., Vichinsky, E. P., & Rachmilewitz, E. A. (2013). Non-transfusion-dependent thalassemias. *Haematologica*, 98(6), 833–844. https://doi.org/10.3324/haematol.2012.075282
30. Origa, R. (2016). β-Thalassemia. *Genetics in Medicine*, 18(6), 441–450. https://doi.org/10.1038/gim.2015.128
31. Origa, R., Galanello, R., Ganz, T., Giagu, N., Maccioni, L., Faa, G., & Nemeth, E. (2007). Liver iron concentrations and urinary hepcidin in beta-thalassemia. *Haematologica*, 92(5), 583–588. https://doi.org/10.3324/haematol.10842
32. Pan, Y., Du, R., Han, X., Zhu, W., Peng, D., Tu, Y., Han, J., Bao, Y., & Yu, H. (2023). Machine learning prediction of iron deficiency anemia in Chinese premenopausal women 12 months after sleeve gastrectomy. *Nutrients*, 15(15), 3385. https://doi.org/10.3390/nu15153385
33. Pearson, H. A., Cohen, A. R., Giardina, P. J., & Kazazian, H. H. (1996). The changing profile of homozygous beta-thalassemia: Demography, ethnicity, and age distribution of current North American patients and changes in two decades. *Pediatrics*, 97(3), 352–356. https://doi.org/10.1542/peds.97.3.352
34. Pepe, A., Gamberini, M., Missere, M., Pistoia, L., Mangione, M., Cuccia, L., Spasiano, A., Maffei, S., Cadeddu, C., Midiri, M., Borgna, C., & Meloni, A. (2018). Gender differences in the development of cardiac complications: A multicentre study in a large cohort of thalassaemia major patients to optimize the timing of cardiac follow-up. *British Journal of Haematology*, 180(4), 563–571. https://doi.org/10.1111/bjh.15125

35. Pinto, V., and Forni, G. L. (2020). Management of iron overload in beta-thalassemia patients: Clinical practice update based on case series. *International Journal of Molecular Sciences, 21(22), 8658*. https://doi.org/10.3390/ijms21228658
36. Premawardhena, A., Mettananda, S., Wijenayake, W., Thennakoon, R., Pathiraja, H., Bandara, D., De Silva, R., & Fernando, M. (2024). Liver and renal complications in patients with beta-thalassaemia: Current perspectives and clinical management. *Sri Lanka Journal of Child Health*, 53(4), 412–420. https://doi.org/10.4038/sljch.v53i4.11234
37. Rumaney, M. B., Ngo Bitoungui, V. J., Vorster, A. A., Ramesar, R. S., Kengne, A. P., Ngogang, J., & Wonkam, A. (2014). The co-inheritance of alpha-thalassemia and sickle cell anemia is associated with better hematological indices and lower consultations rate in Cameroonian patients. *PLOS ONE*, 9(6), e100956. https://doi.org/10.1371/journal.pone.0100956
38. Shah, F. T., Huey, K., Deshpande, S., Turner, M., Chitnis, M., Schiller, E., Yucel, A., Bueno, L., & Olíva, E. N. (2022). Relationship between serum ferritin and outcomes in β-thalassemia: A systematic literature review. *Journal of Clinical Medicine*, 11(15), 4448. https://doi.org/10.3390/jcm11154448
39. Shen, C., Zhang, J., & Wang, L. (2021). Evaluation of the analytical performance of the MAGLUMI X3 automated chemiluminescence immunoassay analyzer. Journal of Clinical Laboratory Analysis, 35(6), Article e23790. https://doi.org/10.1002/jcla.23790.
40. Soliman, A. T., De Sanctis, V., Yassin, M., & Elsedfy, H. (2019). Endocrine complications of thalassemia: An update. *Expert Review of Hematology*, 12(3), 145–163. https://doi.org/10.1080/17474086.2019.1573668
41. Spasiano, A., Meloni, A., Costantini, S., Quaia, E., Cademartiri, F., Cinque, P., Pepe, A., & Ricchi, P. (2021). Setting for "normal" serum ferritin levels in patients with transfusion dependent thalassemia: Our current strategy. *Journal of Clinical Medicine*, 10(24), 5859. https://doi.org/10.3390/jcm10245859
42. Taneri, P. E., Gómez-Ochoa, S. A., Llanaj, E., Raguindin, P. F., Rojas, L. Z., Wyssmann, B., Kopp-Heim, D., Hautz, W. E., Eisenga, M. F., Franco, O. H., Glisic, M., & Muka, T. (2020). Anemia and iron metabolism in COVID-19: A systematic review and meta-analysis. *European Journal of Epidemiology*, 35(8), 763–773. https://doi.org/10.1007/s10654-020-00678-5
43. Tuo, Y., Li, Y., Li, Y., Ma, J., Yang, X., Wu, S., Jin, J., & He, Z. (2024). Global, regional, and national burden of thalassemia, 1990–2021: A systematic analysis for the Global Burden of Disease Study 2021. *eClinicalMedicine*, 72, 102274. https://doi.org/10.1016/j.eclinm.2024.102274

44. Wijenayake, W., Thennakoon, R., Pathiraja, H., Bandara, D., De Silva, R., Premawardhena, A., Fernando, M., & Mettananda, S. (2025). Hepatic and renal functions of paediatric patients with thalassaemia: A cross sectional study from two large thalassaemia centres in Sri Lanka. *BMJ Open*, 15(2), e089784. https://doi.org/10.1136/bmjopen-2024-089784
45. Wyatt, J., Fernando, S., Powell, S., Hill, C., Arshad, I., Probert, C., Ahmed, S., & Hapangama, D. (2023). The role of iron in the pathogenesis of endometriosis: A systematic review. *Human Reproduction Open*, 2023(1), hoad033. https://doi.org/10.1093/hropen/hoad033
46. Yang, W. P., Chang, H. H., Li, H. Y., Lai, Y. L., Huang, T. Y., Tsai, K. S., and Shih, S. R. (2020). Iron overload associated endocrine dysfunction leading to lower bone mineral density in thalassemia major. *The Journal of Clinical Endocrinology and Metabolism, 105(1), e123–e132*. https://doi.org/10.1210/clinem/dgz102