Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Association of Vitamin D3 Levels with Biochemical Disturbances in Beta-Thalassemia Major

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Received: 20/02/2026
Accepted: 10/05/2026
Published: 30/06/2026

Keywords: Beta-Thalassemia Major, Vitamin D3, Serum Ferritin and Iron Overload

DOI:10.62472/kjps.v17.i28.608-617

Abstract

Background: Ferritin and vitamin D are important biochemical markers that reflect iron status and metabolic health, respectively. Their levels may be influenced by demographic and clinical factors such as age, gender, and disease duration. Understanding these relationships is essential for improving disease assessment and management. Objective: This study aimed to evaluate the demographic and clinical characteristics of the study population and to investigate the relationship between gender, age, disease duration, and serum levels of ferritin and vitamins.

Methods: A cross-sectional study was conducted on 48 participants. Demographic and clinical data, including age, gender, disease duration, and family history, were collected. Serum ferritin and vitamin D (D3) levels were measured and analyzed. Statistical analysis was performed using appropriate tests, including descriptive statistics and correlation analysis. A p-value of<0.05 was considered statistically significant.

Results: The study population consisted predominantly of females (60.4%), with the majority of participants aged between 8 and 30 years (91.7%). The majority of patients had a disease duration of 8–30 years (93.8%) and a positive family history (87.5%). Ferritin levels were higher in males (3000 ± 2267) compared to females (2043 ± 2409), while vitamin D levels were higher in females (21.60 ± 25.72) than males (13.80 ± 14.70). However, these differences were not statistically significant ($p >$ XIII 0.05). A strong positive correlation was observed between age and disease duration ($r = 0.923$, $p = 0.001$). No significant correlations were found between age and ferritin or vitamin D levels.

Conclusion: The findings indicate that gender is not significantly associated with variations in ferritin and vitamin D levels. Age was significantly correlated only with disease duration, while no meaningful relationships were observed with biochemical parameters. These results suggest that factors other than age and gender may play a more critical role in influencing ferritin and vitamin D levels. Further studies with larger sample sizes are recommended.

ارتباط مستويات فيتامين د3 بالاضطرابات الكيميائية الحيوية لدى مرضى بيتا- ثلاسيميا الكبرى

أحمد حقي أسماعيل ووليد خالد البهادلي

الخلاصة

خلفية الدراسة: يعد الفيريتين وفيتامين (د) من المؤشرات الحيوية الهامة التي تعكس حالة مخزون الحديد والصحة الأيضية في الجسم. وقد تتأثر مستويات هذه المؤشرات بالعوامل الديموغرافية والسريرية مثل العمر، والجنس، ومدة المرض. لذا، فإن فهم هذه العلاقات يعد أمراً جوهرياً لتطوير آليات تقييم الأمراض وإدارتها.

الهدف: هدفت هذه الدراسة إلى تقييم الخصائص الديموغرافية والسريرية لمجتمع الدراسة، واستقصاء العلاقة بين الجنس والعمر، ومدة المرض، وبين مستويات الفيريتين وفيتامين (د) في المصل.

المنهجية: أُجريت دراسة مقطعية (Cross-sectional) شملت 48 مشاركاً، حيث جُمعت البيانات الديموغرافية والسريرية التي تضمنت العمر، والجنس، ومدة المرض، والتاريخ العائلي. كما تم قياس وتحليل مستويات الفيريتين وفيتامين (د3) في المصل. وأُجري التحليل الإحصائي باستخدام الاختبارات المناسبة، بما في ذلك الإحصاء الوصفي وتحليل الارتباط، مع اعتبار القيمة الاحتمالية ($p < 0.05$) مؤشراً على الدلالة الإحصائية.

النتائج: أظهرت النتائج أن الإناث شكلن النسبة الأكبر من مجتمع الدراسة (60.4%)، وكانت الفئة العمرية السائدة بين 8 و30 عاماً (91.7%). كما بلغت مدة المرض لدى معظم المرضى ما بين 8 إلى 30 سنةً (93.8%)، مع وجود تاريخ عائلي إيجابي لدى أغلبهم (87.5%). سُجلت مستويات فيريتين أعلى لدى الذكور مقارنة بالإناث، بينما كانت مستويات فيتامين (د) أعلى لدى الإناث، ومع ذلك، لم تكن هذه الفروق ذات دلالة إحصائية ($p > 0.05$). وُجد ارتباط طردي قوي بين العمر ومدة المرض ($r = 0.923, p = 0.001$)، في حين لم يظهر أي ارتباط معنوي بين العمر ومستويات الفيريتين أو فيتامين (د).

الاستنتاج: تشير النتائج إلى أن الجنس لا يرتبط بشكل وثيق بالتباين في مستويات الفيريتين وفيتامين (د). كما لم يرتبط العمر معنوياً إلا بمدة المرض، دون وجود علاقات جوهرية مع المؤشرات الحيوية المقاسة. وتوحي هذه النتائج بأن هناك عوامل أخرى غير العمر والجنس قد تلعب دوراً أكثر حيوية في التأثير على مستويات الفيريتين وفيتامين (د).

1. Introduction

The word “thalassemia” derives from the Latin words “thalassia” (great ocean) and “haima” (blood). This disorder is one of the most frequently inherited diseases in the world. Due to its relationship to increased mortality and morbidity in affected individuals, the international health community has placed significant attention on this disorder. There are two types of thalassemias, alpha and beta. Both types are caused by genetic mutations that result in low levels or absent production of either an alpha (α) or beta (β) globin protein. There are cases in which one protein is entirely missing. Globin chains from both the α and β globins are responsible for forming a “globin fold” around the iron (Fe++) heme group which allows the transportation of oxygen throughout the body. These mutations are inherited through an autosomal co-dominant pattern, and single nucleotide polymorphisms (SNPs) and deletions at the gene loci of the globin chains cause the lack of production of the associated hemoglobin (Hb) product, which is termed thalassemia. Thalassemia ultimately results in severe anemia. The alpha and beta globin genes are located on the clusters of chromosomes 16 and 11, respectively. The globin genes expressed change at different points in life as well. For example, in early embryonic and fetal development the alpha globin protein is produced with the gamma globin protein, but after production of the gamma protein, beta globin will eventually take its place. Imbalanced globin chain production can cause hemolytic disease and interfere with the production of red blood cells (Shafique et al., 2023). The majority of patients with Thalassemia require regular blood transfusions to survive and these transfusions are related to many complications of health and other diseases. (Shafique et al., 2023) Among the many complications associated with being a thalassemic, the most dangerous one is the abnormal storage of excess iron in the body, which damages all major organs. There are significant physical and treatment-related challenges for thalassemics because of their disease. Therapeutic challenges associated with thalassemia can be addressed by the timely availability of essential medications and by having a secure system in place to facilitate blood donations. Improving access to specialists is also necessary to provide for people living with this condition. (Kaur, 2023).

2. Patients and Methods

2.1.Study Design

Observational, Analytical, Cross-Sectional Study for Association of Serum Vitamin D3 Levels with Biochemical Disturbances in Patients with Beta-Thalassemia Major: A Cross-Sectional Study in Al-Husseini Hospital in the Holy City of Karbala (Hereditary blood disease center) This design captures the correlation between serum 25(OH)D levels and mineral metabolism at a specific clinical point. The study was accomplished from September 2025 to 20th January 2026 at Karbala, Al-Husseini Hospital in the Holy City /Hereditary Blood Disease Center.

2.2.Study Collected Data

This study was conducted as a cross-sectional clinical investigation at the Hereditary blood disease Al-Husseini Hospital in the holy city of Karbala, Iraq. Data were systematically extracted from the documented medical records of patients diagnosed with Beta-Thalassemia Major. To ensure data integrity and uniformity, a standardized case report form was utilized for all participants. The study parameters focused on key clinical and biochemical indicators, specifically: Patient age, sex, Diagnosis of disease, Duration of the disease, Serum 25-hydroxyvitamin D3 levels (ng/mL)., Serum ferritin levels (ng/mL). All laboratory results were retrieved from finalized, verified clinical reports within the hospital database.

2.3.Inclusion Criteria

Patients with a confirmed diagnosis of Beta-Thalassemia Major receiving care at the Hereditary Hematology Department. 17 Patients with complete documented medical records, including verified laboratory results for serum Vitamin D3 and ferritin levels.

2.4.Exclusion Criteria

The following cases were excluded from the study Incomplete data / Missing biochemical parameters" Diagnostic uncertainty / Lack of confirmed diagnosis. "Supplementation status / Confounding variables"

Statistical Analysis The raw data for the current study was gathered from the laboratory's sheet of laboratory animals and analysed using the IBM SPSS statistical program (version 26). The Shapiro-Wilk test was conduct to assess normality. Non-parametric statistics were employed (Mann-Whitney U test) when assessing the possible association between the related variables of the current study. As the data were non-normally distributed, they are presented as medians with interquartile ranges (IQR). In order to assess the relationships between the variables under investigation, Spearman's rank correlation coefficient (rho) was used. A statistical association was considered to be significant if the associated probability (p) value was less than 0.05 (P value<0.05) or 0.01 (P value<0.01).

2.5.Ethics approval

This study was approved by the Karbala Health Directorate number 2224 in 21 July 2025. Given its retrospective and non-interventional nature, direct patient contact was not required. All data were anonymized at the source to ensure strict patient confidentiality and privacy.

3. Results

The goal of the present research study was to explore the demographic and clinical characteristics of the sample, and also to examine the relationship between gender, age, disease duration and specific biochemical parameters such as Ferritin and Vitamin D levels. This section outlines the results of the statistical analysis conducted on the data collected for this project. These results are organised into three main sections: The first part presents the demographic and clinical characteristics of the sample participants. The second section presents the results of the comparison of Ferritin and Vitamin D levels between male and female subjects. The third section describes the correlation between age and selected variables (disease duration, Ferritin and Vitamin D levels). All data were presented using appropriate statistical measures including frequency distributions, percentage distributions, mean values, standard deviations, correlation coefficients (rho) and p-values. The p-value for determining statistical significance was set at <0.05.

Table1 presents the demographic and clinical characteristics of the study population, including gender, age distribution, disease duration, and etiology. With respect to gender, the results show that females constituted the majority of the study sample, accounting for 29 participants (60.4%), while males represented 19 participants (39.6%). Females constituted a larger proportion of the study sample (60.4%) than males (39.6%). However, this finding only reflects the gender distribution of the recruited participants and should not be interpreted as a higher prevalence of beta-thalassemia major among females. Regarding age distribution, the majority of participants were within the age group of 8–30 years, comprising 44 individuals (91.7%). In contrast, only 4 participants (8.3%) fell within the 31–52 years age group. This suggests that the condition is more commonly observed among younger individuals in the studied sample. In terms of disease duration, most patients reported a duration ranging between 8–30, representing 45 cases (93.8%), whereas only 3 patients (6.3%) had a longer disease duration of 31–52. This finding indicates that the majority of cases were relatively recent or of shorter duration. Concerning etiology, a large proportion of participants (42, 87.5%) had a positive family history (relative), while only 6 cases (12.5%) were classified as having no familial relation (stranger). This highlights the potential role of genetic or familial factors in the occurrence of the condition. Overall, the data demonstrate that the study population was predominantly female, young in age, with shorter disease duration, and largely associated with a familial background. These characteristics may provide important insights into the epidemiological pattern of the condition within this sample.

Table1: Demographic and Clinical Characteristics of The Study Population (N = 48)

Variable	Category	n (%)
Gender	Male	19 (39.6)
	Female	29 (60.4)
Age (years)	8–30	44 (91.7)
	31–52	4 (8.3)
Disease Duration (years)	8–30	45 (93.8)
	31–52	3 (6.3)
Etiology	Relative	42 (87.5)
	Stranger	6 (12.5)

Table2 presents the comparison of ferritin and vitamin D (D3) levels between male and female participants in the study. The findings demonstrate that the mean ferritin level was higher in males (3000 ± 2267) compared to females (2043 ± 2409). This difference may suggest a tendency toward increased iron storage levels in males. However, statistical analysis revealed that this variation was not significant ($p = 0.307$), indicating that gender does not have a significant impact on ferritin levels within the studied population. Regarding vitamin D levels, the results showed that females had a higher mean value (21.60 ± 25.72) compared to males (13.80 ± 14.70). This observation could reflect potential differences in lifestyle, dietary habits, or sun exposure between genders. Nevertheless, this difference was also not statistically significant ($p = 0.302$), suggesting that the observed variation occurred by chance rather than representing a true biological difference. It is important to note that the relatively large standard deviation values in both ferritin and vitamin D measurements indicate considerable variability among participants, which may have

contributed to the lack of statistically significant differences between the groups Overall, although there are observable numerical differences between males and females in both ferritin and vitamin D levels, these differences did not reach statistical significance. Therefore, it can be concluded that gender is not significantly associated with variations in ferritin and vitamin D levels in this study.

Table2: Comparison of Biochemical Parameters Between Male and Female Patients

Parameter	Male (Mean ± SD)	Female (Mean ± SD)	P
Ferritin	3000 ± 2267	2043 ± 2409	0.307
Vitamin D (D3)	13.80 ± 14.70	21.60 ± 25.72	0.302
values are presented as mean ± standard deviation (SD). Differences between males and females were considered statistically significant at $P < 0.05$.			

Table3 presents the correlation between age and selected study variables, including duration of disease, ferritin level, and vitamin D (D3) level, using the correlation coefficient (rho) and corresponding p-values. The results showed a strong positive correlation between age and duration of disease (r = 0.923), which was statistically significant (p = 0.001). This indicates that as age increases, the duration of the disease also tends to increase significantly. This finding is expected, as older individuals are more likely to have lived longer with the disease. In contrast, the correlation between age and ferritin level was negative and weak (r = -0.196), and this association was not statistically significant (p = 0.181). This suggests that although there is a slight tendency for ferritin levels to decrease with increasing age, this relationship is not meaningful from a statistical perspective. Similarly, the correlation between age and vitamin D (D3) level was found to be weak and positive (r = 0.188), but also not statistically significant (p = 0.201). This indicates that age does not have a significant effect on vitamin D levels in the study population. Overall, the findings demonstrate that age is significantly associated only with disease duration, while no significant relationships were observed between age and either ferritin or vitamin D levels.

Table3: Correlation Between Age and Study Variables

Variable	ρ	Strength	P-value
Disease duration	0.923	Very strong positive	0.001*
Ferritin level	−0.196	Weak negative	0.181
Vitamin D_3 level	0.188	Weak positive	0.201
Spearman's rank correlation coefficient (ρ) was used. P < 0.05 indicates statistical significance.			

4. Discussion

The present study aimed to evaluate the demographic and clinical characteristics of the study population and to investigate the relationship between gender, age, disease duration, and selected biochemical parameters, namely ferritin and vitamin D levels. The findings provide important insights into the epidemiological and biochemical patterns of the disease within the studied sample .The results demonstrated that females constituted the majority of the study population compared to males, Females represented a larger proportion of the recruited sample; however, this finding reflects the characteristics of the study population and should not be interpreted as evidence of a higher

disease prevalence among females, immune response differences, and genetic susceptibility (Regitz-Zagrosek, 2012; Klein and Flanagan, 2016). In addition, females are known to exhibit stronger immune reactivity, which may predispose them to certain inflammatory or metabolic conditions (Whitacre, 2001). Regarding age distribution, the majority of participants were within the younger age group, indicating that the disease may be more prevalent or more frequently diagnosed in younger individuals. This finding aligns with studies suggesting that early-life exposure to environmental triggers and genetic predisposition contributes significantly to disease onset (Halfon and Hochstein, 2002; Gluckman and Hanson, 2004). Furthermore, most patients had a relatively short disease duration, reflecting that the study population largely consisted of recently diagnosed cases. This may influence biochemical findings and limit the detection of chronic disease complications. The high proportion of participants with a positive family history further emphasizes the importance of genetic factors in disease development, as supported by previous literature highlighting familial clustering in similar conditions (Cooper and Stroehla, 2003; Ramos and Shedlock, 2015).Regarding biochemical parameters, the study showed that ferritin levels were higher in males compared to females, whereas vitamin D levels were higher in females; however, these differences were not statistically significant. Ferritin is widely recognized as a marker of iron storage and is influenced by multiple physiological and pathological factors, including inflammation and nutritional status (Ganz and Nemeth, 2015). The higher ferritin levels observed in males may be explained by increased iron stores and the absence of menstrual blood loss, which typically reduces iron levels in females (Milman, 2011). Additionally, ferritin is an acute-phase reactant that may increase in response to inflammation, further complicating its interpretation (Kell and Pretorius, 2014). Despite these considerations, the lack of statistical significance suggests that gender alone is not a strong determinant of ferritin levels in this population. Similarly, although females exhibited higher vitamin D levels, the difference was not statistically significant. Vitamin D status is influenced by various factors such as sun exposure, dietary intake, skin pigmentation, and lifestyle habits (Holick, 2007; Bikle, 2010). The large variability observed in vitamin D levels among participants may have masked potential differences between genders. Previous studies have reported inconsistent findings regarding gender differences in vitamin D levels, with some indicating higher levels in females and others reporting no significant association (Palacios and Gonzalez, 2014; van Schoor and Lips, 2011). The correlation analysis revealed a strong positive relationship between age and disease duration, which was statistically significant. This finding is expected, as increasing age is naturally associated with longer exposure to disease, and it supports the internal consistency of the data (Porta, 2014). In contrast, no significant correlations were observed between age and ferritin or vitamin D levels. The weak negative correlation between age and ferritin suggests a possible decline in iron stores with advancing age, although this relationship was not statistically meaningful. This finding may be influenced by confounding factors such as nutritional status, chronic inflammation, and comorbidities (Beard, 2001). Similarly, the weak positive correlation between age and vitamin D levels was not significant, indicating that age alone may not be a reliable predictor of vitamin D status. Previous studies have shown conflicting results, with some reporting decreased vitamin D levels in older individuals due to reduced skin synthesis and others finding no clear association (Mithal et al., 2009; Lips, 2006). From a clinical perspective, the absence of significant differences in ferritin and vitamin D levels between genders suggests that these biomarkers should be interpreted cautiously and not solely based on gender. Instead, a comprehensive evaluation that includes nutritional status, environmental exposure, and disease-related factors is

recommended (Ross et al., 2011). Moreover, the lack of association between age and these biochemical markers indicates that other determinants may play a more critical role in influencing their levels. The strong association between age and disease duration highlights the importance of early diagnosis and intervention to prevent disease progression and long-term complications (World Health Organization, 2014).Despite these findings, several limitations should be acknowledged. The relatively small sample size may have limited the statistical power of the study, reducing the ability to detect significant associations. Additionally, the high variability in ferritin and vitamin D levels may have influenced the results. The absence of data on important confounding factors such as dietary intake, supplementation, and sun exposure represents another limitation. Therefore, future studies with larger sample sizes and better control of confounding variables are necessary to validate these findings and provide more robust conclusions (Sedgwick, 2014).

Conclusion: The findings indicate that gender is not significantly associated with variations in ferritin and vitamin D levels. Age was significantly correlated only with disease duration, while no meaningful relationships were observed with biochemical parameters. These results suggest that factors other than age and gender may play a more critical role in influencing ferritin and vitamin D levels. Further studies with larger sample sizes are recommended.

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