JOURNAL OF BIOMEDICINE AND BIOCHEMISTRY
Volume 4 Issue 2 (2025), pp. 21–39

REVIEW A R T I C A L

Journal JBB
Print ISSN:3006-0532
Online ISSN:2958-0528

# Analysis of the Relationship between NLRP3, Ferritin, MDA, and Catalase Enzyme in Patients with Beta-Thalassemia Major: A Clinical Study

**Ali Abd Al-Bari Al-Dhalimi[1,*] and Zainab N. Al-Abady[2]**

[1]*Faculty of Nursing, University of Babylon, Babylon, 51002, Iraq.*
[2]*Faculty of Pharmacy, University of Babylon, Babylon,51002, Iraq.*

**Corresponding Author Email:**
sci.chem.mas.23.7@qu.edu.iq

**Received:** 19 April 2025,
**Revised:** 22 May 2025,
**Accepted:** 1 June 2025,
**DOI**: 10.57238/jbb.2025.7432.1137

OPEN ACCESS

**Access this article online**



## ABSTRACT

**Background:** The thalassemia syndromes are a group of inherent hemolytic anemias characterized by the decrease or absence of the synthesis of one or more globin chains of hemoglobin. Elevated levels of NOD-like receptor protein 3 (NLRP3) and ferritin, along with oxidative stress markers such as malondialdehyde (MDA) and catalase activity, have been linked to thalassemia disease progression and prognosis. **Aim:** This study aims to investigate the relationship between serum levels of NLRP3, ferritin, MDA, and catalase activity in beta-thalassemia patients compared to healthy controls, providing insights into potential diagnostic and prognostic biomarkers. **Materials and Methods:** (75) patients with beta-thalassemia major aged 6-18 years who were selected at the Al-Zahra Teaching Hospital/ALNAJAF AL ASHRAF CITY along with (45) normal people of the same age without any history of hematological diseases, chronic diseases, acute illness, or infection. Patients who met the selection criteria were included in the study. All subjects were enrolled in this study between November 2024 and January 2025; Serum levels of NLRP3, ferritin, MDA, and Catalase were analyzed using enzyme-linked immunosorbent assay (ELISA) and spectrophotometric methods. For data collection and statistical analysis, Microsoft Office Excel 2013 and GraphPad Prism 9.2.0 were utilized. **Results:** The study found elevated levels of NLRP3 in thalassemia patients (7.006 ± 1.014) compared to the healthy group (1.35 ± 0.2868), with a P value of 0.0003. Ferritin levels were also higher in patients (1576 ± 85.09 ng/ml) compared to the healthy group (73.1 ± 13.67 ng/ml), with a P value of <0.0001. MDA levels increased in patients (0.157 ± 0.01964) compared to the healthy group (0.06813 ± 0.0149), with a highly significant P value of 0.0007. In contrast, catalase activity was significantly lower in the patient group (0.5321 ± 0.07339) than in the healthy group (0.8746 ± 0.1104), with a P value of 0.0129. These findings suggest that an increase in NLRP3, ferritin, and MDA levels and a decrease in catalase activity are associated with an increased risk of thalassemia disease. **Conclusion**: Elevated NLRP3, ferritin, and MDA levels, alongside reduced catalase activity, are associated with thalassemia disease progression. These biomarkers could serve as valuable tools for early detection and monitoring therapeutic responses in thalassemia patients.

**Keywords:** Irritable bowel syndrome (IBS); Lifestyle factors; Dietary habits.

## 1. Introduction

Thalassemia is caused by mutations in the genes that encode the alpha- or beta-globin chains of adult hemoglobin (Hb), which are referred to as alpha-thalassemia and beta-thalassemia, respectively. The disease's distinguishing feature is the resulting alpha/non-alpha-globin chain imbalance, which causes inefficient erythropoiesis and peripheral hemolysis, eventually leading to chronic hemolytic anemia. The severity of anemia and the requirement for transfusion therapy are strongly related to the underlying

genotype, although many molecular and environmental variables remain at play (1). Beta-thalassemia is a group of inherited blood diseases characterized by anomalies in hemoglobin beta chain synthesis, which can result in a wide variety of phenotypes, from severe anemia to clinically asymptomatic individuals. The global annual incidence of symptomatic people is estimated at one in 100,000, with one in 10,000 in the European Union.

Thalassemia is classified into three types: major, intermediate, and minor (2). Individuals with thalassemia major usually develop severe anemia in their first two years of life, necessitating regular red blood cell (RBC) transfusions. Individuals with thalassemia major who are untreated or poorly transfused exhibit growth retardation, pallor, jaundice, weak musculature, hepatosplenomegaly, leg ulcers, the formation of masses from extramedullary hematopoiesis, and skeletal abnormalities caused by bone marrow expansion (2). Several treatment options exist, including hydroxyurea or other drugs to modulate gamma-globulin chain synthesis, transfusion, splenectomy, and stem cell transplant. Many of these individuals need iron chelation therapy, even if they have not been transfused (3). Patients with beta-thalassemia who rely on transfusions encounter a variety of difficulties as a result of their permanent need for packed red blood cells. These include blood-borne infections, iron excess, the toxicities of iron chelation, and bacterial infections (4). Furthermore, frequent blood donations can lead to iron overload, which can cause hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism, and other endocrine disorders (5). Iron excess can result in serious problems, such as cardiac disease and osteopathy. In the second decade of life, myocardial iron overload leads to cardiomyopathy and heart failure mortality. severe liver damage, leading to cirrhosis and hepatocellular cancer (6).

Multiple blood transfusions, insufficient erythropoiesis, and increased gastrointestinal iron absorption all contribute to the body's iron overload. Iron overload impairs the immune system, making patients more susceptible to infection and disease. The serum ferritin levels can be used to assess iron overload (7). Progressive iron deposition disrupts and destroys multiple organs, including the bone marrow, heart, liver, and glands, such as the hypophysis, thyroid, parathyroid, suprarenal, and pancreas. Circulating iron surplus can directly raise the amounts of blood ferritin (an iron-binding molecule) (8). Ferritin is a diagnostic marker of iron overload in thalassemia patients, increasing due to inadequate erythropoiesis, iron overload, and transfusion (8). The ferritin serum concentration is an indirect sign of iron overload(9). Iron overload in thalassemia patients results in oxygen-free radicals and peroxidative lipid damage. MDA, a molecule generated during lipid peroxidation, may signal oxidative stress (9). Oxidative stress has a significant role in the progression of β-thalassemia major and other disorders, including cardiovascular failure, cancer, renal and neurological diseases, infections, and more. This process is distinguished by increased metabolic generation of reactive oxygen species (ROS) and enhanced lipid peroxidation (LPO). Oxidative stress surpasses the capacity of antioxidant defenses (antioxidant concentration and enzyme activity). In patients with β-thalassemia major, it causes oxidation of both internal and surface components of red blood cells (10). A variety of endogenous and external defensive systems can neutralize and prevent the harmful effects of labile iron and its related reactive compounds. Endogenous antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, and ferroxidase, may directly or progressively end the activity of ROS (11). Catalase was first discovered by Louis Jacques Thenard in 1818. It is an intracellular enzyme made up of four polypeptide chains that include four porphyrin heme groups. Catalase is responsible for detoxifying hydrogen peroxide within cells (12).

Inflammasomes are multimeric protein complexes that are assembled by intracytoplasmic pattern recognition receptors. They play an important role in innate immunity. Jurg Tcholop first proposed them

in 2002. Macrophages, dendritic cells, neutrophils, and epithelial cells all contribute to the formation of the inflammasome (13). When the body is invaded by pathogenic microorganisms or injured by endogenous danger signals, pattern-recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), induce the assembly of the inflammasome, and promote the release of the proinflammatory cytokines interleukin (IL)-1β and IL-18, the occurrence of an immune response, and pyroptosis, which exerts significant positive effects on immune defense (13). NLRs serve an important function in detecting chemicals associated with intracellular infection and stress. Thus, they detect a number of general stimuli that suggest intracellular microbial infection and damage, including variations in ion concentrations and the formation of reactive oxygen species (ROS) (14). The NLRP3 inflammasome in the innate immune system activates caspase-1 and releases proinflammatory cytokines IL-1β/IL-18 in response to cellular damage or microbial infection. Several inflammatory disorders, including cryopyrin-associated periodic syndromes, Alzheimer's disease, diabetes, and atherosclerosis, have been linked to abnormal activation of the NLRP3 inflammasome over time (12). This study aims to investigate the relationship between serum levels of NLRP3, ferritin, MDA, and catalase activity in beta-thalassemia patients compared to healthy controls, providing insights into potential diagnostic and prognostic biomarkers.

## 2. Materials and Methods

### 2.1. Study Population

(75) patients with beta-thalassemia major aged 6-18 years were chosen at the Al-Zahra Teaching Hospital/ALNAJAF AL ASHRAF CITY, along with (45) healthy people of the same age who had no history of hematological disorders, chronic diseases, acute sickness, or infection. Patients who met the selection criteria were included in the study. All subjects were enrolled in this study between November 2024 and January 2025; they signed a written informed consent form from their father or mother for child patients. The study was ethically approved by Najaf Health Directorate (reference letter number 37235 on 05.11.2024).

### 2.2. Methods

Blood samples (5 ml) were collected from 120 participants. The samples were divided into sections: the blood was collected in a gel tube and centrifuged at 3600 rpm for 10-15 minutes to separate the serum. The serum was then divided and stored in Eppendorf tubes at -20°C for later analysis of NLRP3, and the remaining serum was used for biochemical analysis (MDA levels and catalase activity). The NLRP3 level was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) method, and the ferritin level was measured by laboratory tests using the i-star500 device from Drawray Biotech in China. The iStar-ferritin assay is a sandwich immunoassay using a direct chemiluminescence technique. The iStar-ferritin assay includes the following steps:

1. **Incubation**: Ferritin in the sample reacts with anti-ferritin-coated superparamagnetic microparticles and acridinium-labeled anti-ferritin conjugate to form a sandwich (antibody-antigen-antibody) complex.
2. **Washing**: Under a magnetic field, the magnetic microparticles are adsorbed to the wall of the reaction vessel, and unbound materials are washed away by the wash buffer.

3. **Signal triggering and measuring**: The pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs).
4. The amount of Ferritin in the sample is proportional to RLUs measured by the analyzer's optical systems.
5. Results are determined via a calibration curve: The calibration curve is generated by 3-point calibration, and a master curve is provided via the QR code**.**

### 2.3. Inclusion Criteria

All patients of both genders (6-18 years) met the criteria for thalassemia in Iraq. and the diagnosis was confirmed by clinical features, biochemical tests, and hematological criteria.

### 2.4. Exclusion criteria

Patients with other blood diseases and those with any infection or chronic inflammation, congestive heart and liver failure, as well as chronic disease, were excluded from the study.

### 2.5. Statistical Analysis

For data collection and statistical analysis, Microsoft Office Excel 2013 and GraphPad Prism 9.2.0 were utilized. The data are presented as mean ± standard error of the mean (SEM). For variables that were normally distributed, group means were compared using Chi-Square, t-test, one-way ANOVA, and Pearson correlation (r). A p-value of less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. Measurement of Serum Malondialdehyde

The patient group showed significantly higher MDA concentrations (0.157 ± 0.01964) compared to the healthy group (0.06813 ± 0.0149), with a highly significant P value of 0.0007. This indicates a significant increase in oxidative stress in patients, as MDA is a byproduct of lipid peroxidation and a marker of oxidative damage. As shown in Figure 1.

Table 1 compares the mean levels of malondialdehyde (MDA) between healthy and patient groups, stratified by gender.

**Table 1.** Comparing the average MDA levels between the healthy group and the patient group according to gender.

| Characteristic | Healthy | | Patients | | P-value |
|---|---|---|---|---|---|
| | Male | Female | Male | Female | |
| | ***n*=14** | ***n*=16** | ***n*=33** | ***n*=27** | |
| **Range** | 0.003365 - 0.1481 | 0.000942 - 0.2625 | 0.008346 - 0.2692 | 0.009154 - 0.2692 | 0.0015 |
| **Mean ± SEM** | 0.06884 ± 0.01475 | 0.06747 ± 0.02587 | 0.1746 ± 0.02051 | 0.1351 ± 0.02109 | ** |

***n***: number of cases; *: significant P < 0.05

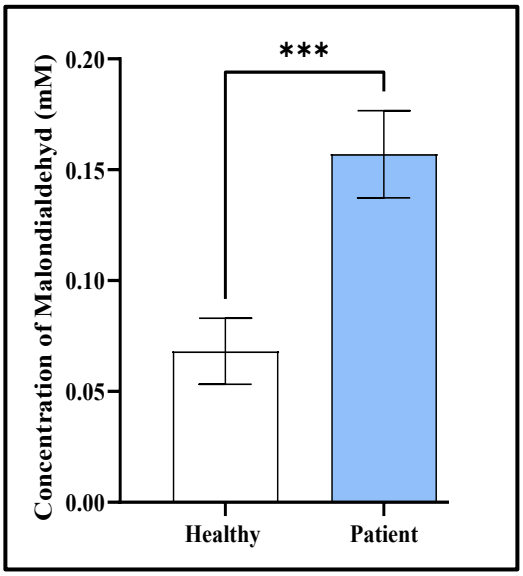


**Figure 1.** A comparison of mean serum malondialdehyde (MDA) between the healthy group and the patient group shows a significant difference. The figures show a highly significant (p-value = 0.0007) difference in the patient's group as compared with the healthy. Data are expressed as means ± SEM. Indicates *significant P≤0.05.

There is a significant increase in MDA levels in the patient group compared to the healthy group. Additionally, within the patient group, males have significantly higher MDA levels than females, indicating a potential gender-based difference in oxidative stress. As shown in Figure 2.

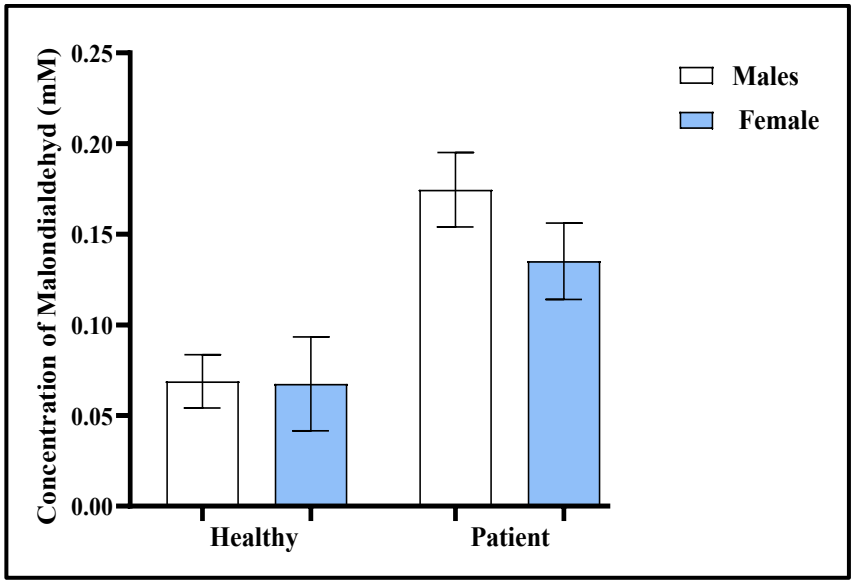


**Figure 2.** A comparison of mean serum malondialdehyde (MDA) between the healthy group and the patient group shows a significant difference. The figures show a highly significant (p-value = 0.0007) difference in the patient's group as compared with the healthy. Data are expressed as means ± SEM. Indicates *significant P≤0.05.

Table 2 provides the results of Tukey's multiple comparisons test for MDA levels, comparing the mean serum MDA concentrations between healthy and patient groups based on gender.

**Table 2.** Tukey's multiple comparisons of mean serum MDA levels between healthy and patient groups based on gender.

| Tukey's multiple comparisons test | Summary | Adjusted P-Value |
|---|---|---|
| Healthy (Male vs. Female) | ns | >0.9999 |
| Healthy Male vs. Male patients | ** | 0.0079 |
| Healthy Male vs. Female patients | ns | 0.1694 |
| Healthy Female vs. Male patients | ** | 0.0054 |
| Healthy Female vs. Female patients | ns | 0.1399 |
| Patient (Male vs. Female) | ns | 0.4833 |

***ns:* No significant; **:* significant P = <0.05**

The Tukey's multiple comparisons test reveals significant differences in MDA levels between healthy males and male patients, as well as between healthy females and male patients. However, no significant gender differences were observed within the healthy group or among female patients, suggesting that MDA levels are more influenced by health status than gender in this context.

Table **3** compares the serum MDA levels between healthy and patient groups based on age categories (6-12 years and >13 years).

**Table 3.** Age-Based Comparison of Serum MDA levels between healthy and patient groups.

| Characteristic | Healthy | | Patients | | P-value |
|---|---|---|---|---|---|
| | 6-12 years | >13 years | 6-12 years | >13 years | |
| | *n*=13 | *n*=17 | *n*=23 | *n*=37 | |
| **Range** | 0.000942 - 0.138 | 0.003365 - 0.2625 | 0.008346 - 0.2692 | 0.008346 - 0.2579 | 0.0024 |
| **Mean ± SEM** | 0.04778 ± 0.01473 | 0.08412 ± 0.02357 | 0.1546 ± 0.02458 | 0.155 ± 0.01887 | ** |

***n*:** number of cases; ***:** Significant = <0.05

This table demonstrates significant differences in serum MDA levels based on age within both the healthy and patient groups. In the healthy group, MDA levels tend to increase with age, while in the patient group, MDA levels are significantly higher across both age categories compared to the healthy group, suggesting that oxidative stress is elevated in patients regardless of age. As shown in Figure 3.

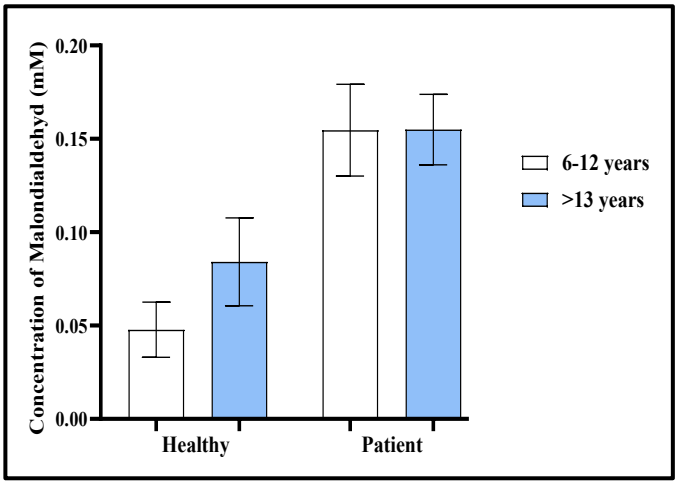


**Figure 3.** Comparison of mean serum MDA levels between healthy and patient groups based on age.

### 2.7. Measurement of serum Catalase Activity

Catalase activity was found to be significantly lower in the patient group (0.5321 ± 0.07339) than in the healthy group (0.8746 ± 0.1104), with a P value of 0.0129. This suggests that the patient group has a reduced antioxidant defense capacity, as catalase is an enzyme that neutralizes reactive oxygen species. As shown in Figure 4.

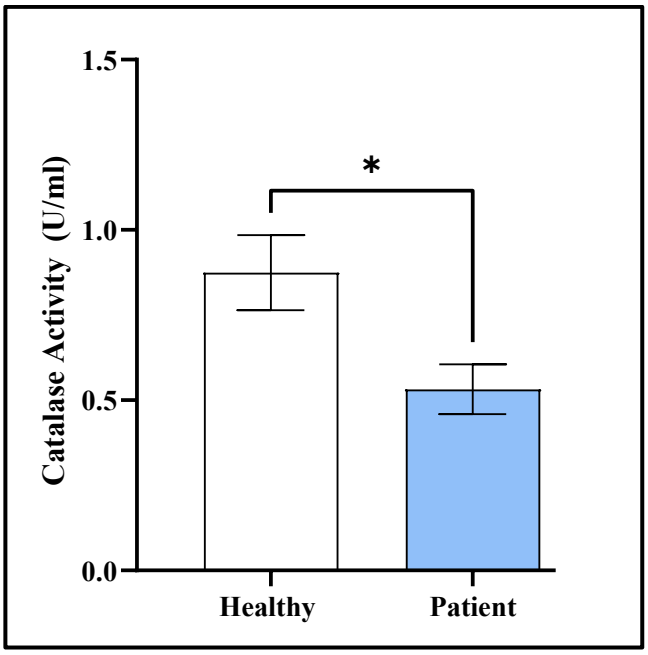


**Figure 4.** A comparison of mean serum catalase activity between the healthy group and the patient group shows a significant difference. The figures show a highly significant (p-value = 0.0129) difference in the patient's group as compared with healthy. Data are expressed as means ± SEM. Indicates *significant P≤0.05.

Table 4 compares the catalase activity levels between healthy and patient groups, stratified by gender.

**Table 4.** Comparing the average Catalase Activity levels between the healthy group and the patient group according to gender.

| Characteristic | Healthy | | Patients | | P-value |
|---|---|---|---|---|---|
| | Male | Female | Male | Female | |
| | *n*=14 | *n*=16 | *n*=33 | *n*=27 | |
| Range | 0.3058 - 1.682 | 0.1529 - 2.294 | 0.1529 - 0.9174 | 0.1529 - 1.988 | 0.0063 |
| Mean ± SEM | 0.7518 ± 0.09681 | 0.988 ± 0.1917 | 0.474 ± 0.04948 | 0.5428 ± 0.09112 | ** |

***n*:** number of cases; ***:** Significant = <0.05

The table highlights significant gender-based differences in catalase activity, with females exhibiting higher catalase activity than males in both the healthy and patient groups. The patient group, regardless of gender, shows a notable reduction in catalase activity compared to the healthy group, reflecting potential alterations in antioxidant defense mechanisms in patients. As shown in Figure 5.

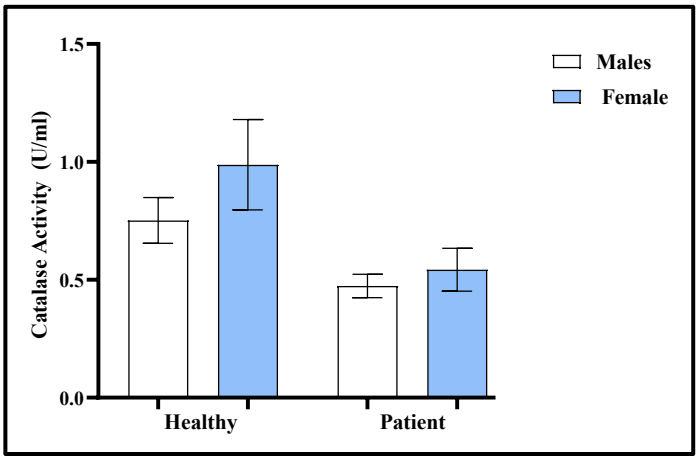


**Figure 5.** Comparison of mean serum Catalase Activity levels between healthy and patient groups stratified by gender.

Table 5 provides the results of Tukey's multiple comparisons test for catalase activity levels, comparing the mean serum catalase concentrations between healthy and patient groups based on gender.

**Table 5.** Comparing the average Catalase Activity levels between the healthy group and the patient group according to gender.

| Tukey's multiple comparisons test | Summary | Adjusted P-Value |
|---|---|---|
| Healthy (Male vs. Female) | ns | 0.5129 |
| Healthy Male vs. Male patients | ns | 0.2895 |
| Healthy Male vs. Female patients | ns | 0.5388 |
| Healthy Female vs. Male patients | ** | 0.0066 |
| Healthy Female vs. Female patients | * | 0.0237 |
| Patient (Male vs. Female) | ns | 0.9561 |

***n*:** number of cases; ***:** Significant = <0.05

Tukey's multiple comparisons test reveals that there are significant gender-based differences in catalase activity when comparing healthy females with female patients and when comparing healthy females with male patients. However, there are no significant differences within the healthy group, between males and females in the patient group, or between healthy males and patient groups, indicating that catalase activity is predominantly influenced by health status rather than gender in these comparisons.

Table **6** compares the serum catalase activity levels between healthy and patient groups based on age categories (6-12 years and >13 years).

**Table 6.** Comparing the average Catalase Activity levels between the healthy group and the patient group according to gender.

| Characteristic | Healthy | | Patients | | P-value |
|---|---|---|---|---|---|
| | 6-12 years | >13 years | 6-12 years | >13 years | |
| | *n*=13 | *n*=17 | *n*=23 | *n*=37 | |
| **Range** | 0.3058 - 2.294 | 0.1529 - 1.835 | 0.1529 - 1.988 | 0.1529 - 0.9174 | 0.0013 |
| **Mean ± SEM** | 1.098 ± 0.1976 | 0.699 ± 0.106 | 0.5307 ± 0.1058 | 0.492 ± 0.04606 | ** |

***n*:** number of cases; ***:** Significant P= <0.05

This table demonstrates significant differences in serum catalase activity based on age within both the healthy and patient groups. In the healthy group, younger individuals (6-12 years) exhibit higher catalase activity compared to older individuals (>13 years). In the patient group, catalase activity is reduced across both age categories compared to the healthy group, with a slight decrease in activity as age increases. These findings suggest that oxidative stress, reflected by catalase activity, may be more pronounced in older individuals within the patient group. As shown in Figure 6.

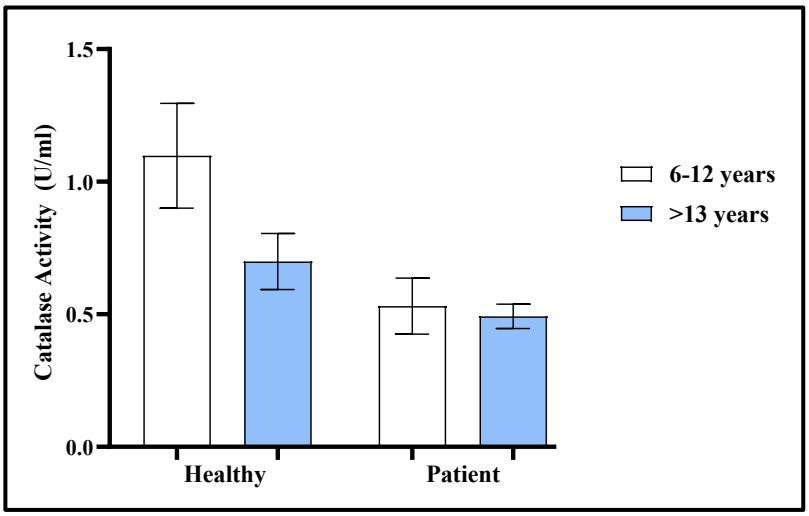


**Figure 6.** Comparison of mean serum Catalase Activity levels between healthy and patient groups based on age.

### 2.8. Measurement of Serum NLRP3

A significant elevation in NLRP3 levels was observed in the patient group (7.006 ± 1.014) compared to the healthy group (1.35 ± 0.2868), with a P value of 0.0003. This suggests that NLRP3 may play a crucial role in the inflammatory or immune response in the patient group. As shown in Figure 7.

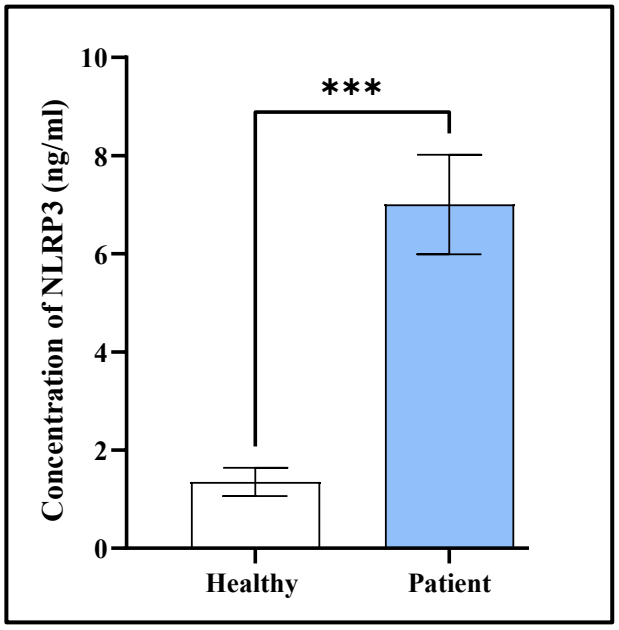


**Figure 7.** A comparison of mean serum Nod-like receptor pyrin-3 (NLRP3) between the healthy

group and the patient group shows a significant difference.

The figures show a highly significant (p-value = 0.0003) difference in the patient's group as compared with the healthy. Data are expressed as means ± SEM. Indicates *significant P≤0.05.

Table 7 compares the NLRP3 levels between the healthy and patient groups, stratified by gender.

**Table 7.** Comparing the average Catalase Activity levels between the healthy group and the patient group according to gender.

| Characteristic | Healthy | | Patients | | P-value |
|---|---|---|---|---|---|
| | Male | Female | Male | Female | |
| | *n*=14 | *n*=16 | *n*=33 | *n*=27 | |
| Range | 0.038 - 8.662 | 0.3916 - 2.111 | 0.7316 - 20.09 | 0.8312 - 46.47 | 0.0026 |
| Mean ± SEM | 1.574 ± 0.6091 | 1.156 ± 0.1212 | 6.134 ± 0.8233 | 8.073 ± 2.022 | ** |

***n*:** number of cases; ***:** Significant P= <0.05

The table reveals that NLRP3 levels significantly differ between genders in both the healthy and patient groups. In the healthy group, males exhibit higher NLRP3 levels than females. However, in the patient group, females show significantly higher NLRP3 levels than males. These results suggest a potential gender-related variation in NLRP3 levels, with a notable increase in NLRP3 levels observed in females within the patient group. As shown in Figure 8.

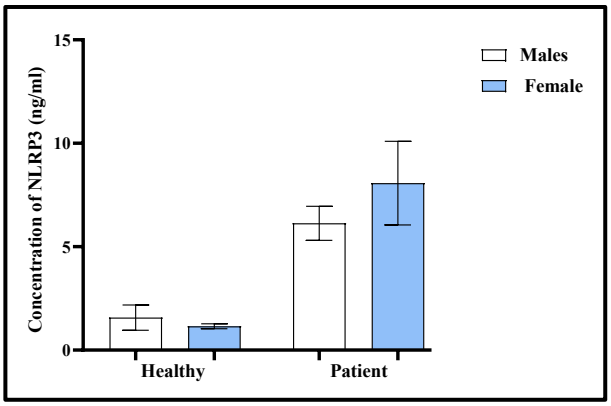


**Figure 8.** Comparison of mean serum NLRP3 levels between healthy and patient groups stratified by gender.

Table 8 presents the results of Tukey's multiple comparisons test for the mean serum NLRP3 levels between the healthy and patient groups, broken down by gender.

**Table 8.** Tukey's multiple comparisons of mean serum NLRP3 levels between healthy and patient groups based on gender.

| Tukey's multiple comparisons test | Summary | Adjusted P-Value |
|---|---|---|
| Healthy (Male vs. Female) | ns | 0.9983 |
| Healthy Male vs. Male patients | ns | 0.1574 |
| Healthy Male vs. Female patients | * | 0.0228 |
| Healthy Female vs. Male patients | ns | 0.08 |
| Healthy Female vs. Female patients | ** | 0.0086 |
| Patient (Male vs. Female) | ns | 0.6698 |

***n*:** number of cases; ***:** Significant P= <0.05

Tukey's multiple comparisons test shows significant differences in NLRP3 levels between certain subgroups. Notably, female patients have significantly higher NLRP3 levels compared to healthy females, while male patients do not show significant differences when compared to healthy males. These results suggest that gender may play a role in NLRP3 levels, with a notable difference observed in females within the patient group.

Table **9** compares the serum NLRP3 levels between the healthy and patient groups, categorized by age.

**Table 9.** Age-Based Comparison of Serum NLRP3 levels between healthy and patient groups.

| Characteristic | Healthy | | Patients | | P-value |
|---|---|---|---|---|---|
| | 6-12 years | >13 years | 6-12 years | >13 years | |
| | *n*=13 | *n*=17 | *n*=23 | *n*=37 | |
| **Range** | 0.7474 - 8.662 | 0.038 - 1.473 | 0.7548 - 27.41 | 0.7316 - 46.47 | 0.0038 |
| **Mean ± SEM** | 1.884 ± 0.5808 | 0.8873 ± 0.112 | 7.689 ± 1.568 | 6.582 ± 1.337 | ** |

***n*:** number of cases; ***:** Significant P= <0.05

The table shows a significant difference in serum NLRP3 levels based on age within both the healthy and patient groups. In both groups, the 6-12 years age group has significantly higher serum NLRP3 levels compared to the >13 years group. This suggests that age may influence NLRP3 levels, with younger individuals showing higher levels in both healthy and patient populations. As shown in Figure 9.

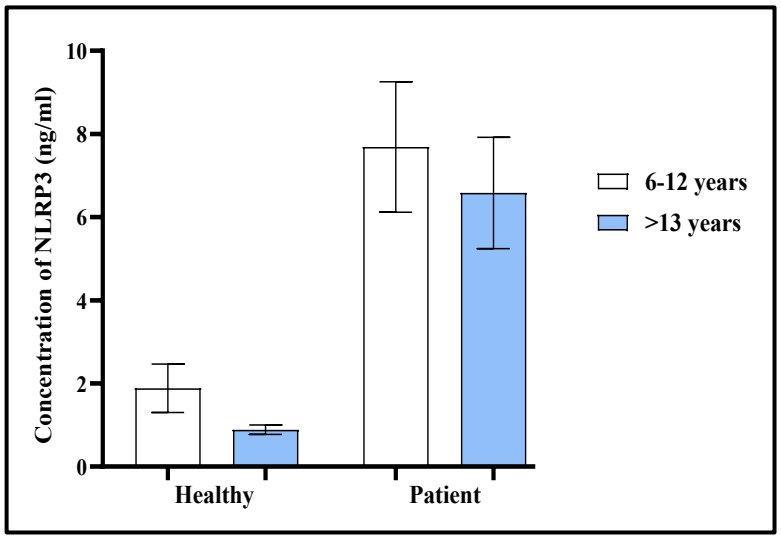


**Figure 9.** Comparison of mean serum NLRP3 levels between healthy and patient groups based on age.

### 2.9. Measurement of Serum Ferritin

Ferritin levels were significantly elevated in the patient group (1576 ± 85.09 ng/ml) compared to the healthy group (73.1 ± 13.67 ng/ml), with a P value of <0.0001. The marked increase in ferritin suggests an inflammatory response, as ferritin is an acute-phase reactant and may reflect iron metabolism dysregulation in patients. As shown in Figure 10.

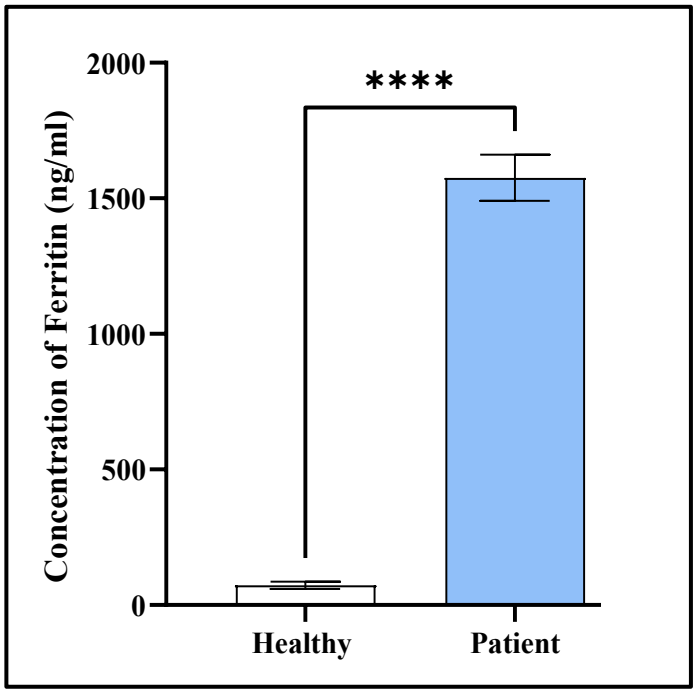


**Figure 10.** Comparison of mean serum ferritin between the healthy group and the patient group shows a significant difference.

The figures show a highly significant (*p-value* < 0.0001) difference in the patient's group as compared with the healthy. Data are expressed as means ± SEM. Indicates *significant P≤0.05.

Table **10** compares the serum ferritin levels between the healthy and patient groups, categorized by gender.

**Table 10.** Comparing the average ferritin levels between the healthy group and the patient group according to gender.

| Characteristic | Healthy | | Patients | | P-value |
|---|---|---|---|---|---|
| | Male | Female | Male | Female | |
| | ***n*=14** | ***n*=16** | ***n*=33** | ***n*=27** | |
| **Range** | 24.4 - 300 | 24.4 - 121.7 | 664-2780 | 422-2202 | <0.0001 |
| **Mean ± SEM** | 81.82 ± 21.89 | 60.91 ± 8.936 | 1594 ± 119 | 1550 ± 125.7 | **** |

***n*:** number of cases; ***:** Significant P= <0.05

The analysis indicates that ferritin levels are significantly elevated in the patient group compared to the healthy group for both males and females. This suggests that elevated ferritin levels may be associated with the condition of the patients and may serve as an important biomarker for the disease in both genders. As shown in Figure 11.

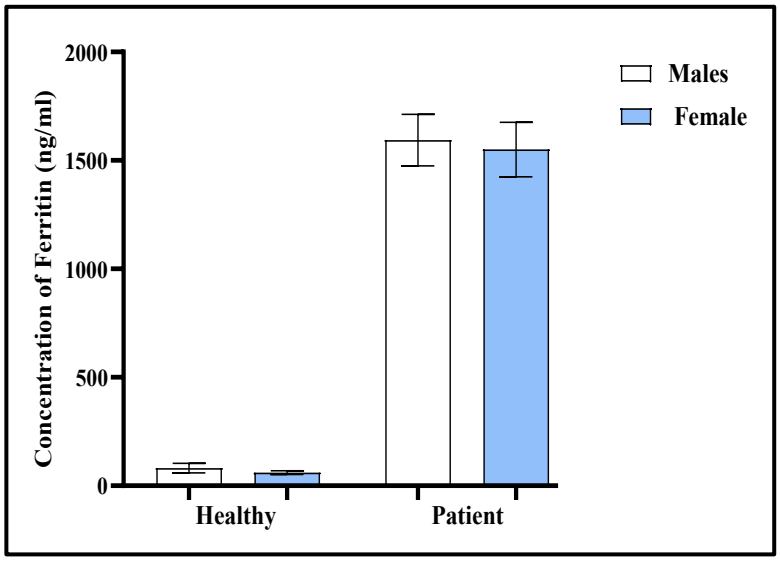


**Figure 11.** Comparison of mean serum ferritin levels between healthy and patient groups stratified by gender.

Table 11 presents the results of Tukey's multiple comparisons test to assess the differences in mean serum ferritin levels between the healthy and patient groups, separated by gender.

**Table 11.** Comparing the average ferritin levels between the healthy group and the patient group according to gender.

| Tukey's multiple comparisons test | Summary | Adjusted P-Value |
|---|---|---|
| Healthy (Male vs. Female) | ns | 0.9994 |
| Healthy Male vs. Male patients | **** | <0.0001 |
| Healthy Male vs. Female patients | **** | <0.0001 |
| Healthy Female vs. Male patients | **** | <0.0001 |
| Healthy Female vs. Female patients | **** | <0.0001 |
| Patient (Male vs. Female) | ns | 0.9888 |

***n*:** number of cases; ***:** Significant P= <0.05

Tukey's multiple comparisons test highlights significant differences in ferritin levels between healthy and patient groups across both genders. Notably, both male and female patients exhibit markedly higher ferritin levels than their healthy counterparts, emphasizing the potential role of ferritin as a biomarker for the disease. However, no significant difference is observed between male and female patients.

Table 12 compares the serum ferritin levels based on age groups (6-12 years and >13 years) between healthy and patient groups.

**Table 12.** Comparing the average ferritin levels between the healthy group and the patient group according to gender.

| Characteristic | Healthy | | Patients | | P-value |
|---|---|---|---|---|---|
| | 6-12 years | >13 years | 6-12 years | >13 years | |
| | *n*=13 | *n*=17 | *n*=23 | *n*=37 | |
| **Range** | 30.2 - 90.2 | 24.4 - 300 | 422-2780 | 495-2100 | <0.0001 |
| **Mean ± SEM** | 46.31 ± 5.658 | 90.3 ± 18.6 | 1745 ± 137.9 | 1444 ± 102.8 | **** |

***n*:** number of cases; ***:** Significant P= <0.05

The data indicate that serum ferritin levels are significantly higher in patients compared to healthy individuals in both age categories. Ferritin levels in patients, particularly those aged 6-12 years, show marked elevation, further suggesting that ferritin may serve as a reliable marker for the condition. The significant difference in ferritin levels across all age groups highlights its potential as a diagnostic tool.

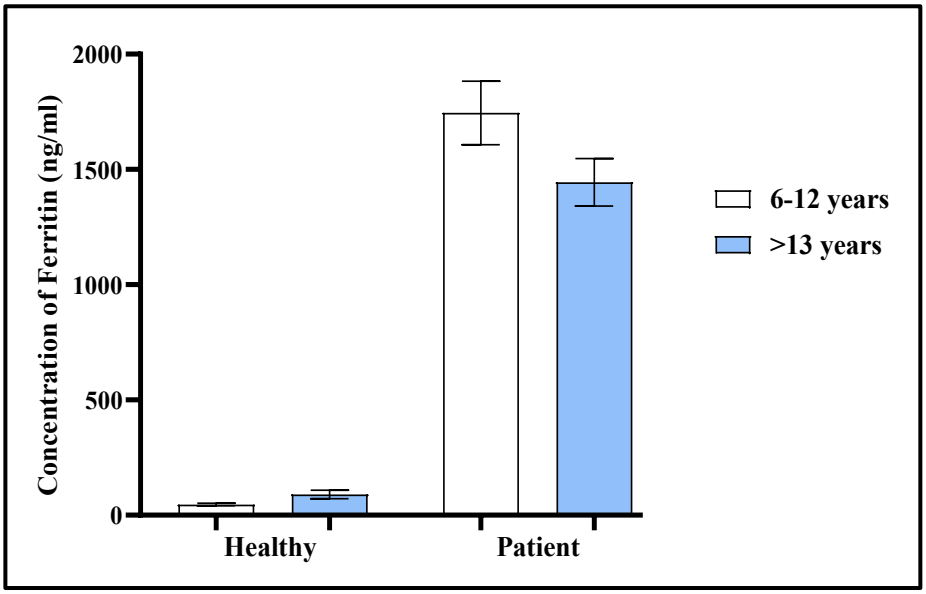


**Figure 12.** Comparison of mean serum ferritin levels between healthy and patient groups based on age.

### 2.10. Correlation

#### 2.10.1. NLRP-3 and other variables

NLRP-3 shows a moderate positive correlation with S. Ferritin ($r = 0.576$, p-value $< 0.0001$), suggesting a significant relationship. Other variables like MDA and CAT show weak or no correlations with NLRP-3.

#### 2.10.2. S. Ferritin and other variables

S. Ferritin shows a significant positive correlation with MDA ($r = 0.233$, p-value = 0.148), but the p-value is just above the threshold for significance (0.05), suggesting a potential relationship that requires further examination in larger studies.

#### 2.10.3. MDA and Other Variables

MDA shows weak or no significant correlation with other biological markers. The highest correlation is with S. Ferritin ($r = 0.233$), but the relationship is not significant (p-value = 0.148).

## 4. Discussion

The patient group showed significantly higher MDA concentrations ($0.157 \pm 0.01964$) compared to the healthy group ($0.06813 \pm 0.0149$), with a highly significant P value of 0.0007. This indicates a significant increase in oxidative stress in patients, as MDA is a byproduct of lipid peroxidation and a marker of oxidative damage. Increased levels of serum MDA in the thalassemia subjects compared to healthy subjects were caused by the absence of beta-globin chains in beta-thalassemia patients, leading to the accumulation of excess alpha-globin chains. The presence of excess alpha-globin chains was a primary cause of cellular oxidative damage as well as iron overload. As a result of increased levels of plasma iron

and non-hemoglobin intracellular iron in thalassemia, the production of ROS will be increased(15). Excessive iron in thalassemia patients causes peroxidative damage to tissues, raised MDA levels, and ROS production. Iron overload is caused primarily by inefficient erythropoiesis, which results in severe anemia, with blood transfusions serving as a secondary influence. As a result, higher iron concentrations may make red blood cells and other cellular constituents more vulnerable to oxidative damage(16). Catalase activity was found to be significantly lower in the patient group (0.5321 ± 0.07339) than in the healthy group (0.8746 ± 0.1104), with a P value of 0.0129. This suggests that the patient group has a reduced antioxidant defense capacity, as catalase is an enzyme that neutralizes reactive oxygen species. Oxidative stress is defined as the interruption of balance between oxidants and reductants within the body due to the excess production of peroxides and free radicals. During the course of metabolism, superoxide anion is converted to $H_2O_2$ by the ubiquitous enzyme SOD. Normally, $H_2O_2$ is converted to innocuous compounds by the action of catalase and peroxidase. But if free iron is available, it reacts with $H_2O_2$ to form hydroxyl radicals, which are extremely reactive species leading to depolymerization of polysaccharides, DNA strand breakage, inactivation of functional proteins, and other events(15). The results of this study were consistent with previous studies. Kósa et al. revealed a significant decrease in catalase activity of 43 β-thalassemia carriers and attributed it to catalase protein damage by increased free radicals and $H_2O_2$ (17).

A study found that persons with β-thalassemia minor had higher amounts of antioxidant enzymes such as SOD, catalase, and GPx in their red blood cells, while those with β-thalassemia major had near-normal levels. They concluded that β-thalassemia minor RBCs react to increased OxS rising activities of antioxidant enzymes, while in β-thalassemia major, normal antioxidant enzyme levels are due to the presence of normal RBCs from multiple blood transfusions (18). Ferritin levels were significantly elevated in the patient group (1576 ± 85.09 ng/ml) compared to the healthy group (73.1 ± 13.67 ng/ml), with a P value of <0.0001. The marked increase in ferritin suggests an inflammatory response, as ferritin is an acute-phase reactant and may reflect iron metabolism dysregulation in patients. Ferritin is a key indicator of iron levels in thalassemia patients.

However, it can be impacted by inflammation and illness consequences(19). The current study's findings revealed a substantial difference between the two groups in terms of ferritin levels. In a similar vein, Saraya Ak. et al. did a study to test serum ferritin in thalassemia patients and reported an increase in ferritin(20). John C et al. also documented a ferritin rise in their research of cardiac iron overload in transfusion-dependent patients(21). In general, this iron overload is caused by two mechanisms: blood transfusion and inadequate erythropoiesis (22). In thalassemia, the GDF15 protein, which is the result of mutations in such patients, works as the inhibitor of the peptide-hepcidin hormone and sends its reducing signal to the liver. Following hepcidin reduction, iron absorption from the diet is increased by ferroportin. Therefore, deficient erythrocytes built in the spleen are trapped, resulting in iron release, which eventually leads to an increase in ferritin (20-22).A significant elevation in NLRP3 levels was observed in the patient group (7.006 ± 1.014) compared to the healthy group (1.35 ± 0.2868), with a P value of 0.0003. This suggests that NLRP3 may play a crucial role in the inflammatory or immune response in the patient group. In this study, we found that excess cellular labile iron activates the NLRP3 inflammasome, leading to secretion of IL-1β in human monocytes. The generation of ROS has been considered to be an upstream event for NLRP3 inflammasome activation in response to a wide range of stimuli(23, 24). Our results raise the possibility that NLRP3 inflammasome activation might be involved in the production of pro-inflammatory cytokines in patients with iron overload(25). Given that iron deposition in the reticuloendothelial system is commonly observed in patients with iron overload, NLRP3 inflammasome activation might occur in reticuloendothelial cells that are exposed to locally high concentrations of iron. Because the inflammasome plays a critical role in inflammation-associated organ fibrosis(26, 27).

Our study showed significant relationships between NLRP3 and ferritin levels. We consistently found

that cellular labile iron activates the NLRP3 inflammasome through ROS-dependent mitochondrial dysfunction(28). underlining the significance of iron in inflammatory responses. However, the potential that excess cellular iron could be detected by an intracellular danger sensor, the inflammasome, has not before been investigated. Iron-mediated NLRP3 inflammasome activation, along with the hepcidin-ferroportin axis, may form a positive feedback loop that enhances inflammation, as IL-1β boosts hepatocyte hepcidin synthesis. Further research is needed to better understand the interaction between the hepcidin-ferroportin axis and NLRP3 inflammasome activation by cellular iron(29). Iron-mediated organ dysfunction is a main cause of the mortality and morbidity in patients with thalassemia major, which causes iron overload as a result of increased intestinal iron absorption and/or transfusion dependency(30). Our study also found a correlation between ferritin and MDA levels. MDA and ferritin levels are markedly elevated in both transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT). MDA may be utilized as a surrogate marker to assess iron overload in beta-thalassemia. Iron overload and oxidative stress can occur in beta-thalassemia in the absence of regular blood transfusions (31).

Our work is unique in that NLRP3 levels were investigated for the first time in thalassemia patients. Because there have been no previous studies on this topic, we were unable to locate results in literature reviews on NLRP3 levels in thalassemia patients. In our investigation, we discovered that these individuals have high NLRP3, ferritin, and MDA levels, implying that they suffer from chronic inflammation. The clinical implications of this study include revealing NLRP3, ferritin, MDA, and catalase enzyme as key biomarkers with significant diagnostic and therapeutic potential, emerging as valuable indicators for monitoring disease progression and evaluating treatment responses, offering clinicians objective measures for patient assessment. This study identified NLRP3, ferritin, MDA, and the catalase enzyme as possible biomarkers for thalassemia diagnosis and monitoring. However, significant limitations limit clinical translation: The small sample size (75 patients, 45 controls) necessitates validation in larger cohorts, the observational design cannot establish causality between biomarkers and disease progression, and there is no prior research on this specific combination, preventing comparison with existing evidence. While these unique findings point to potential clinical therapies, bigger mechanistic research is required to confirm causal linkages and therapeutic efficacy before implementing them in routine thalassemia management.

## 5. Conclusion

This study underscores the significant biochemical changes associated with thalassemia disease. Elevated levels of NLRP3, ferritin, and MDA highlight increased inflammation and oxidative stress in thalassemia patients. Conversely, reduced catalase (CAT) activity reflects impaired antioxidant defense mechanisms. These findings suggest that NLRP3 and ferritin could serve as valuable prognostic biomarkers for thalassemic disease, while MDA and catalase enzymes provide insights into the oxidative and antioxidative status of patients. Collectively, these biomarkers have potential clinical applications for disease monitoring, risk assessment, and guiding therapeutic strategies in thalassemic disease.

## 6. Limitations of the Study

There were several problems faced in conducting this research, including the difficulty of obtaining samples from patients to conduct the research and the absence of previous research studies on this subject. In addition, the size of the sample collected was small and insufficient, the high financial cost, and the short time constraints.

**Acknowledgments:** The researchers would like to express their gratitude to Al-Qadisiyah University/College of Science/Department of Chemistry and its members, including professors and

laboratory authorities, for the facilities offered. The management and staff of Al-Zahra Teaching Hospital in Najaf al-Ashraf, including doctors and analysts, assisted us in completing this project.

**Conflict of interest statement:** The authors have no conflict of interest with respect to the publication of this article.

**The Authors Involved in the Research:** The research Ali Abd Al-Bari Al-Dhalimi and assistant Professor Dr. Zainab N. Al-Abadi contributed to the research design to analyze the results and write the manuscript, and the authors approved the final version for submission.

**Ethical Consideration:** The ethical committee approved the study at University of Al-Qadisiyah, Al-Qadisiyah, Iraq.

## References

1. Musallam KM, Lombard L, Kistler KD, Arregui M, Gilroy KS, Chamberlain C, et al. Epidemiology of clinically significant forms of alpha-and beta-thalassemia: a global map of evidence and gaps. American journal of hematology. 2023;98(9):1436-51. https://doi.org/10.1002/ajh.27006
2. Galanello R, Origa R. Beta-thalassemia. Orphanet journal of rare diseases. 2010;5:1-15. https://doi.org/10.1186/1750-1172-5-11
3. Karimi M, Cohan N, De Sanctis V, Mallat NS, Taher A. Guidelines for diagnosis and management of Beta-thalassemia intermedia. Pediatric hematology and oncology. 2014;31(7):583-96. https://doi.org/10.3109/08880018.2014.937884
4. Nemtsas P, Arnaoutoglou M, Perifanis V, Koutsouraki E, Orologas A. Neurological complications of beta-thalassemia. Annals of hematology. 2015;94:1261-5. https://doi.org/10.1007/s00277-015-2378-z
5. Shamshirsaz AA, Bekheirnia MR, Kamgar M, Pourzahedgilani N, Bouzari N, Habibzadeh M, et al. Metabolic and endocrinologic complications in beta-thalassemia major: a multicenter study in Tehran. BMC endocrine disorders. 2003;3:1-6. https://doi.org/10.1186/1472-6823-3-4
6. Shawkat AJ, Jwaid AH. Clinical complications of beta-thalassemia major. Iraqi Journal of Pharmaceutical Sciences (P-ISSN 1683-3597 E-ISSN 2521-3512). 2019;28(2):1-8. https://doi.org/10.31351/vol28iss2pp1-8
7. Mishra AK, Tiwari A. Iron overload in Beta thalassaemia major and intermedia patients. Maedica. 2013;8(4):328. PMID: 24790662
8. Saad BH, Abdul-AM A-HH, Hussein A-MB, Mazin J. The study of serum ferritin level as a predictor of growth retardation in Thalassemia-major. Archivos Venezolanos de Farmacologia y Terapeutica. 2021;40(5):492-501.
9. Gunarsih A, Amalia P, Boediman I. Variables associated with malondialdehyde level in thalassemia major patients. Paediatrica Indonesiana. 2012;52(3):125-31.
10. Pavlova L, Savov V, Petkov H, Charova I. Oxidative stress in patients with beta-thalassemia major. Prilozi. 2007;28(1):145-54.
11. Fibach E, Rachmilewitz EA. The role of antioxidants and iron chelators in the treatment of oxidative stress in thalassemia. Annals of the New York Academy of Sciences. 2010;1202(1):10-6. https://doi.org/10.1111/j.1749-6632.2010.05577.x
12. Shazia Q, Mohammad Z, Rahman T, Shekhar HU. Correlation of oxidative stress with serum trace element levels and antioxidant enzyme status in Beta thalassemia major patients: a review of the literature. Anemia. 2012;2012(1):270923. https://doi.org/10.1155/2012/270923

13. Xu X, Wu X, Yue G, An Q, Lou J, Yang X, et al. The role of Nod-like receptor protein 3 inflammasome activated by ion channels in multiple diseases. Mol Cell Biochem. 2023;478(6):1397-410. https://doi.org/10.1007/s11010-022-04602-1
14. Almeida-da-Silva CLC, Savio LEB, Coutinho-Silva R, Ojcius DM. The role of NOD-like receptors in innate immunity. Frontiers in Immunology. 2023;14:1122586. https://doi.org/10.3389/fimmu.2023.1122586
15. Şimşek F, Öztürk G, Kemahlı S, Erbaş D, Hasanoğlu A. Oxidant and antioxidant status in beta thalassemia major patients. Journal of Ankara University Faculty of Medicine. 2005;58(1):34-8.
16. Bou-Fakhredin R, De Franceschi L, Motta I, Eid AA, Taher AT, Cappellini MD. Redox Balance in β-Thalassemia and sickle cell disease: a love and hate relationship. Antioxidants. 2022;11(5):967. https://doi.org/10.3390/antiox11050967
17. Kósa Z, Nagy T, Nagy E, Fazakas F, Góth L. Decreased blood catalase activity is not related to specific beta-thalassemia mutations in Hungary. International Journal of Laboratory Hematology. 2012;34(2):172-8. https://doi.org/10.1111/j.1751-553X.2011.01377.x
18. Gerli G, Beretta L, Bianchi M, Pellegatta A, Agostoni A. Erythrocyte superoxide dismutase, Catalase and glutathione peroxidase activities in β-thalassaemia (major and minor). Scandinavian Journal of Haematology. 1981;25(1):87-92. https://doi.org/10.1111/j.1600-0609.1981.tb01370.x
19. Tantiworawit A, Khemakapasiddhi S, Rattanathammethee T, Hantrakool S, Chai-Adisaksopha C, Rattarittamrong E, et al. Correlation of hepcidin and serum ferritin levels in thalassemia patients at Chiang Mai University Hospital. Bioscience Reports. 2021;41(2):BSR20203352. https://doi.org/10.1042/BSR20203352
20. Saraya AK, Kumar R, Choudhry VP, Kailash S, Sehgal AK. A study of serum ferritin in beta thalassemia: iron deficiency and overload. American journal of clinical pathology. 1985;84(1):103-7. https://doi.org/10.1093/ajcp/84.1.103
21. Wood JC, Tyszka JM, Carson S, Nelson MD, Coates TD. Myocardial iron loading in transfusion-dependent thalassemia and sickle cell disease. Blood. 2004;103(5):1934-6. https://doi.org/10.1182/blood-2003-06-1919
22. Trinder D, Fox C, Vautier G, Olynyk J. Molecular pathogenesis of iron overload. Gut. 2002;51(2):290-5. https://doi.org/10.1136/gut.51.2.290
23. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011;469(7329):221-5. https://doi.org/10.1038/nature09663
24. Martinon F. Signaling by ROS drives inflammasome activation. European journal of immunology. 2010;40(3):616-9. https://doi.org/10.1002/eji.200940168
25. Esposito BP, Breuer W, Sirankapracha P, Pootrakul P, Hershko C, Cabantchik ZI. Labile plasma iron in iron overload: redox activity and susceptibility to chelation. Blood. 2003;102(7):2670-7. https://doi.org/10.1182/blood-2003-03-0807
26. Gieling RG, Wallace K, Han Y-P. Interleukin-1 participates in the progression from liver injury to fibrosis. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2009;296(6):G1324-G31.
27. Artlett CM. The role of the NLRP3 inflammasome in fibrosis. The open rheumatology journal. 2012;6:80. https://doi.org/10.2174/1874312901206010080
28. Rajamaki K, Lappalainen J. Oö rni K, Vä limä ki E, Matikainen S, Kovanen PT, et al. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol

metabolism and inflammation. PLoS One. 2010;5:e11765. https://doi.org/10.1371/journal.pone.0011765
29. Nakamura K, Kawakami T, Yamamoto N, Tomizawa M, Fujiwara T, Ishii T, et al. Activation of the NLRP3 inflammasome by cellular labile iron. Experimental hematology. 2016;44(2):116-24.
30. Rund D, Rachmilewitz E. β-Thalassemia. New England Journal of Medicine. 2005;353(11):1135-46. https://doi.org/10.1016/j.exphem.2015.11.002
31. Basu D, Adhya DG, Sinha R, Chakravorty N. Role of malonaldehyde as a surrogate biomarker for iron overload in the β-thalassemia patient: A systematic meta-analysis. Advances in Redox Research. 2021;3:100017. https://doi.org/10.1016/j.arres.2021.100017

How to cite this article

Al-Dhalimi, A.A.A.; Al-Abady, Z.N. Analysis of the Relationship between NLRP3, Ferritin, MDA, and Catalase Enzyme in Patients with Beta-Thalassemia Major: A Clinical Study. Journal of Biomedicine and Biochemistry. 2025;4(2):21-39. doi: 10.57238/jbb.2025.7432.1137