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Association of adiponectin gene expression and oxidative stress markers with β-thalassemia major patients in Babylon City

T. H. Mgheer

University of Babylon, Babylon, Iraq

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Department of Chemistry and Biochemistry, College of Medicine, University of Babylon, Babylon, Iraq. Email: tariqhussien967@gmail.com

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Beta-thalassemia major (β-TM) is a severe hereditary hematological illness marked by a substantial decrease or total lack of beta-globin chain production, resulting in inefficient erythropoiesis and persistent hemolytic anemia. The objective of this research was to examine the association of gene expression of adiponectin with β-TM disease compared with the internal control gene GAPDH using the qPCR technique. The study also verified the association of oxidative stress with β-TM disease by estimating the levels of MDA and SOD using the ELISA assay. Furthermore, we assessed the levels of clinical biomarkers in the beta-thalassemia major patients (G2) as compared with the control (G1). The case-control study was conducted on 150 participants, 75 of whom were beta-thalassemia major patients (G2) and 75 of whom were healthy controls (G1). The participants' age ranged 7 to 14 years, and all of them were non-smokers and did not suffer from other diseases in order to exclude the resulting harms, thus enhancing the reliability of the results. The clinical examinations were conducted to estimate biomarkers (creatinine, blood urea, total bilirubin, total protein, HCT, HGB, PLT, RBCs, and WBCs) at Babylon Hospital for Women and Children, and the gene expression tests for the adiponectin gene and the ELISA assay to estimate MDA and SOD were conducted at the laboratories of the College of Science, Al-Qadisiyah University. Significant differences were observed between the groups in the adiponectin gene expression, MDA, and SOD levels, and also the biomedical parameters (creatinine, blood urea, total bilirubin, total protein, HCT, HGB, PLT, RBCs, and WBCs). Thus, the gene expression analysis of adiponectin using the GAPDH gene as a reference gene in the qPCR technique showed a decline in the level of gene expression of adiponectin in the β-TM patients (G2) compared with the control (G1). The results of the fold change (2-ΔΔct) were 0.024, and the efficiency of sample amplification ranged 91% to 95%, indicating the quality of the gene expression results. The ELISA measurements of MDA and SOD revealed an increase in their levels, where their mean values were high in the β-TM patients (G2) compared with the control (G1), highlighting a significant difference between the two groups. The study found an increase in the levels of blood urea, total bilirubin, PLT, and WBCs, as the mean values for them were high in G2 compared with G1. At the same time, a decrease was found in the levels of creatinine, total protein, HCT, HGB, and RBCs, as their mean values were low in G2, compared with G1. Also, we observed a reduced adiponectin gene expression. In the study, an increase in MDA levels led to higher SOD levels, suggesting a compensatory response to oxidant stress damage. These indicators can be used to diagnose or monitor β-TM disease.

Keywords: beta-thalassemia; major disease; β-TM; adiponectin; ADIPOQ.

Introduction

The inherited blood disorder beta-thalassemia major (β-TM), often known as Cooley's anemia, causes inefficient erythropoiesis and prolonged hemolytic anemia owing to decreased or missing beta-globin chain synthesis. This condition is caused by mutations in the hemoglobin beta (HBB) gene on chromosome 11 (Ali et al., 2021). Hemoglobin, with four alpha and beta chains, transports oxygen in red blood cells. Due to an imbalance between alpha and beta synthesis, it produces too many unstable alpha-globin chains (Yadav & Singh, 2022). Unbound alpha chains break cell membranes in precursors of bone marrow red blood cells, causing premature death of erythroid cells and ineffective erythropoiesis. Serious anemia, pallor, stunted growth, jaundice, and hepatosplenomegaly affect infants (Rashid & Abbasi, 2020). Chronic anemia causes extramedullary hematopoiesis, which may expand the liver, spleen, and lymph nodes by increasing erythropoietin production and marrow expansion (Baird et al., 2022). The body cannot eliminate 200–250 mg of iron from transfused blood. Iron deposits damage the heart, liver, and endocrine glands, causing their malfunction (Chiew et al., 2021). Heart failure, arrhythmias, and cardiomyopathy lead to the death of most transfused beta-thalassemia major patients. Iron overload may induce cirrhosis and liver failure, whereas endocrine dysfunction can cause diabetes, hypogonadism, hypothyroidism, and hypoparathyroidism (Xu & Wu, 2020). Beta-thalassemia major is treated with lifelong blood transfusions to block inefficient erythropoiesis and extramedullary hematopoiesis and maintain hemoglobin above 9–10 g/dL (Gagliardi et al., 2022). Iron overload requires treatment with deferoxamine, deferiprone, and deferasirox, which promote its elimination (Daar et al., 2023). Splenectomy may be considered in patients with substantial splenomegaly, hypersplenism, or high transfusion demands; nevertheless, it may result in thrombosis and infection. The patient's defective hematopoietic stem cells can only be repaired by BMT or HSCT, which replace the defective cells with healthy stem cells from an appropriate donor, typically a sibling (Musallam et al., 2023). Bone marrow transplantation can promote healing but may also induce graft-versus-host disease, infection, and mortality. New therapeutic options include gene therapy, which introduces a working HBB gene into hematopoietic stem cells (Jaafari et al., 2022). The ADIPOQ gene encodes the adipocyte-derived hormone adiponectin, which is essential for lipid homeostasis, glucose metabolism, and anti-inflammatory processes (Harbi et al., 2020). Research indicates that β-TM patients have lower adiponectin levels, which may be brought on by oxidative stress, iron overload, and chronic inflammation. Patients with β-TM may be more susceptible to diabetes and metabolic syndrome due to dysregulation of adiponectin gene expression (Sarhat et al., 2020). Despite oxidative stress and adipokine dysregulation contributing to β-thalassemia major, the relationship between adiponectin levels, lipid peroxidation markers such as MDA, and antioxidant defences such as SOD is unclear in affected populations. Despite genetic background, dietary state, and environmental exposures influencing biomarker profiles, most research has been done in ethnic or regional groups. Little research has been conducted on patients in Babylon, where β-thalassemia is prevalent and lifestyle variables and

healthcare constraints may affect disease manifestation. This gap urges further study of these biomarkers in this cohort to determine their involvement in the disease development and therapeutic or prognostic potential.

Materials and methods

The study was conducted using a case-control design on 150 participants, who were visiting Babylon Hospital for Women and Children – Genetic Blood Diseases Center in Babylon, Iraq. A total of 75 of them were β-TM patients (G2) and 75 were the healthy controls (G1). The study was authorized by the Ethical Approval Committee of the University of Al-Qadisiyah, College of Science (Reference No. 329, dated January 6, 2025). The collecting of samples from the participants began June 14, 2025, and ended August 28, 2025. The participants' age ranged 7 to 14 years. All of them were non-smokers and did not suffer from liver, heart, kidney, or other comorbidities. Thus, the effect of complications resulting from those conditions was excluded, allowing for the study of beta-thalassemia major only, and more accurately. A total of 5 mL of venous blood was collected from all the participants in the study groups (G1 and G2). The serum was separated by spinning it in a centrifuge for approximately 10 minutes at room temperature (4,000 x g). The serum and whole blood were stored at –20 °C until used for various experiments. Venous blood in an amount of 1 mL was isolated in an Eppendorf tube, and 200 µL of triazole was added to it for the purpose of using it in the study of gene expression of adiponectin. At the same time, 4 mL of the remaining blood was converted to serum to evaluate oxidant stress markers (MDA and SOD) using the ELISA assay and estimate the levels of clinical biomarkers. The study was conducted in three parts. The first segment included specialized assessments of a number of biological parameters, including creatinine, blood urea, total bilirubin, total protein, HCT, HGB, PLT, RBCs, and WBCs, conducted directly using the Beckman Coulter Au 480 instrument (Beckman Coulter, USA). The second part of the study included evaluating the oxidant markers (MDA and SOD) with the ELISA assay by using a Bibase device (Bibase, USA). The third segment was focused on the genetic expression test of adiponectin. The extraction of RNA for analysis was conducted using a 5 mL blood sample obtained from each participant in the two research groups (G1 and G2). Genomic RNA was isolated from blood using a RNA Extraction Kit (ADDBio/Korea) at a concentration of 20 µg/mL or lower. Following RNA extraction, absorbance at 260–280 nm was assessed using a Nanodrop instrument to ascertain RNA concentration and purity. The standard metrics were derived from the outcomes of the Quantus Fluorometer. Subsequently, assessment of the RNA samples was performed with Quantus procedures. The concentrations ranged 10 to 65 ng/µL, and the purity ranged 1.7 to 1.9. The cDNA synthesis was conducted using a Promega (USA) QuantusTM Fluorometer and a kit (ADDBio/Korea). The extracted cDNA was amplified using specified primers. We used specific primers for the genes GAPDH and adiponectin in a real-time qPCR assay. The primers for GAPDH were 5'-GTCTCCTCTGACTTCAACAGCG-3' (forward) and 5'-ACCACCCTGTTGCTGTAGCCAA-3' (reverse), and the primers for adiponectin were 5'-GCAGCTCCTAGCCGTAGACTCTGCTG-3' (forward) and 5'-GGAGGTCTGTGATGAAAGAGGCC-3' (reverse). The assay was performed using a BioRad real-time PCR apparatus. Thermal conditions were conducted for BioRAD (USA). The cDNA derived from total RNA was synthesized with the cDNA synthesis kit (ADDBio/Korea). The Ct values derived from the genomic DNA sample were used to compute the copy numbers for the adiponectin and GAPDH genes. The commonest and the most straightforward method was used to determine the likelihood that both target and reference genes are amplified with the same ~100% efficiency. Before using the Livak approach, it is necessary to check that the target and reference genes have the same amplification efficiencies. The below formula was used to normalize the transcript levels using adiponectin, and GHRL data according to the ΔΔCt method:

$$\Delta CT\ (test) = CT\ (target, test) - CT\ (ref, test),$$
$$\Delta CT\ (control) = CT\ (target, control) - CT\ (ref, control),$$
$$\Delta\Delta CT = \Delta CT\ (test) - \Delta CT\ (control),$$
$$2\text{-}\Delta\Delta CT = [(CT\ gene\ of\ interest - CT\ internal\ control)\ sample\ A - (CT\ gene\ of\ interest - CT\ internal\ control)\ sample\ B)],$$
$$2\text{-}\Delta\Delta CT = ratio\ of\ normalized\ expression,$$
$$E\% = 10^{-1}\ x\ slope\ (Initial\ conc) - 1.$$

According to the descriptive statistics, there was a significant correlation between adiponectin levels and GAPDH gene expression measured by RT-qPCR in the two groups, with a P-value less than 0.05. The results of the study are presented as mean ± standard deviation (SD) or as percentages. For quantitative variables, a T-test in SPSS version 27 (SPSS Inc., Chicago, IL, USA) was used to evaluate the mean differences between the two groups. If the P-value was less than 0.05 at a 95% confidence interval, it was considered statistically significant. GraphPad Prism version 9 was used to conduct a statistical analysis of clinical biological markers and the ELISA measurements of MDA and SOD (GraphPad Software, San Diego, CA, USA). The findings are shown as mean ± standard deviation (SD) and $P \leq 0.05$. The study of the mean ± standard deviation was made possible using the Student's t-test. Statistical significance is indicated by a P-value below 0.05.

Results

Clinical features and ELISA Assay of MDA and SOD in the study groups (G1 and G2). This analysis examined the parameter tests of the control group (G1) and β-TM disease patient group (G2), yielding the data presented in Table 1.

Table 1
Comparison of the parameters in the research groups (G1 and G2)

Parameters	Groups		T-test	P-value
	G1-control mean ± SD	G2-, β-TM mean ± SD		
Creatinine, mg/dL	0.980 ± 0.160	0.481 ± 0.073	23.42	0.009*
B. urea, mg/dL	16.4 ± 2.7	20.5 ± 2.7	18. 52	0.0038*
T. bilirubin, mg/dL	0.68 ± 0.17	2.19 ± 0.05	25.81	0.007*
T. protein, g/dL	7.58 ± 0.77	5.93 ± 0.59	26.64	0.004*
HCT, %	36.9 ± 2.6	16.6 ± 1.0	28.37	0.005*
HGB, g/dL	12.33 ± 2.06	5.81 ± 0.74	42.25	0.0009*
PLT, µL	229 ± 41	487 ± 52	37.86	0.0001*
RBCs, µL	4.65 ± 0.35	2.86 ± 0.54	30.83	0.001*
WBCs, µL	9.64 ± 1.28	19.47 ± 1.72	38.95	0.001*
MDA, nmol/mL	21.3 ± 2.7	37.2 ± 3.4	18.35	0.008*
SOD, U/L	231 ± 28	368 ± 23	27.45	0.001*

Note: T. bilirubin stands for total bilirubin; T. protein for total protein; HCT for hematocrit; HGB for hemoglobin; B. urea for blood urea; PLT for platelet; RBCs for red blood cells; WBCs for white blood cells; the mean ± SD is used to represent the data; a P-value of less than 0.05 is deemed significant.

The data variance was statistically significant when P-values were below 0.05. Table 1 presents the clinical characteristics and ELISA test results for MDA and SOD. This experiment used age matching to reduce discrepancies in the parameter values caused by substantial age variations.

Association of genetic expression of adiponectin gene as compared with housekeeping (GAPDH) gene and risk of β-TM patients' group (G2) compared with the control group (G1). The data in Table 2 are the results of the relative quantification analysis of adiponectin gene expression in the β-TM patient group (G2) and the control group (G1). Figures 1 and 2 compare the amplification of the GAPDH gene in G1 and G2, whereas Figures 3 and 4 compare the amplification of the adiponectin gene in G1 and G2.

Table 2
The comparison between the gene expression of adiponectin (ADIPOQ) gene as compared with housekeeping (GAPDH) gene of the study groups (G1 and G2)

Gene	Groups		T-test	P-value	$2^{-\Delta\Delta ct}$	Efficiency%
	G1-control (Mean ±SD)	G2-, β-TM (Mean ± SD)				
ADIPOQ	27.31 ± 3.58	16.35 ± 1.19	23.57	0.001*	0.024	91–95%

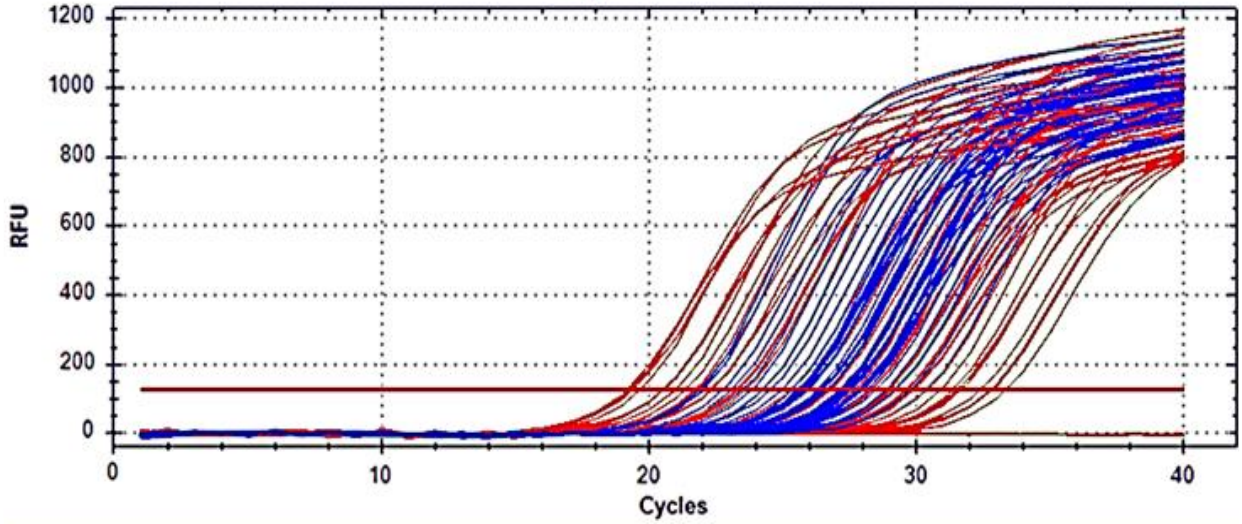

Fig. 1. Amplification curve of the housekeeping (GAPDH) gene expression in the β-TM patient group (G2, red line) and the control individuals (G1, blue line)

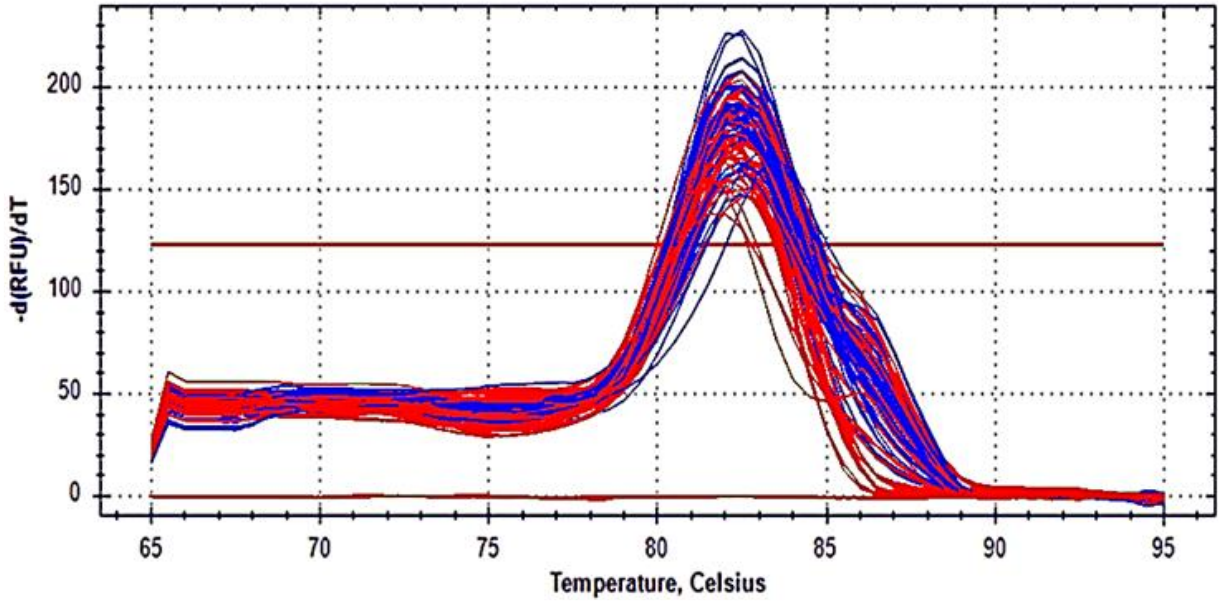

Fig. 2. Disassociation melting curve analysis of the housekeeping (GAPDH) gene expression in the β-TM patient group (G2, red line) and the control group (G1, blue line)

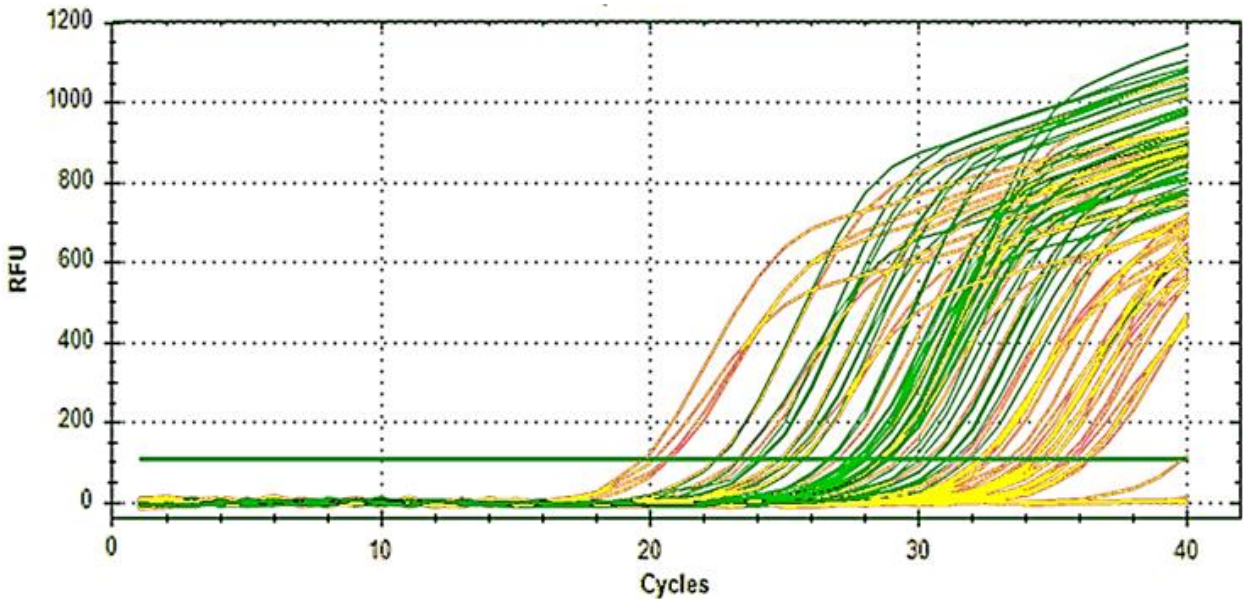

Fig. 3. Amplification curve of the expression of the gene of interest (ADIPOQ) in the β-TM patient group (G2) depicted in yellow: green indicates the control group (G1)

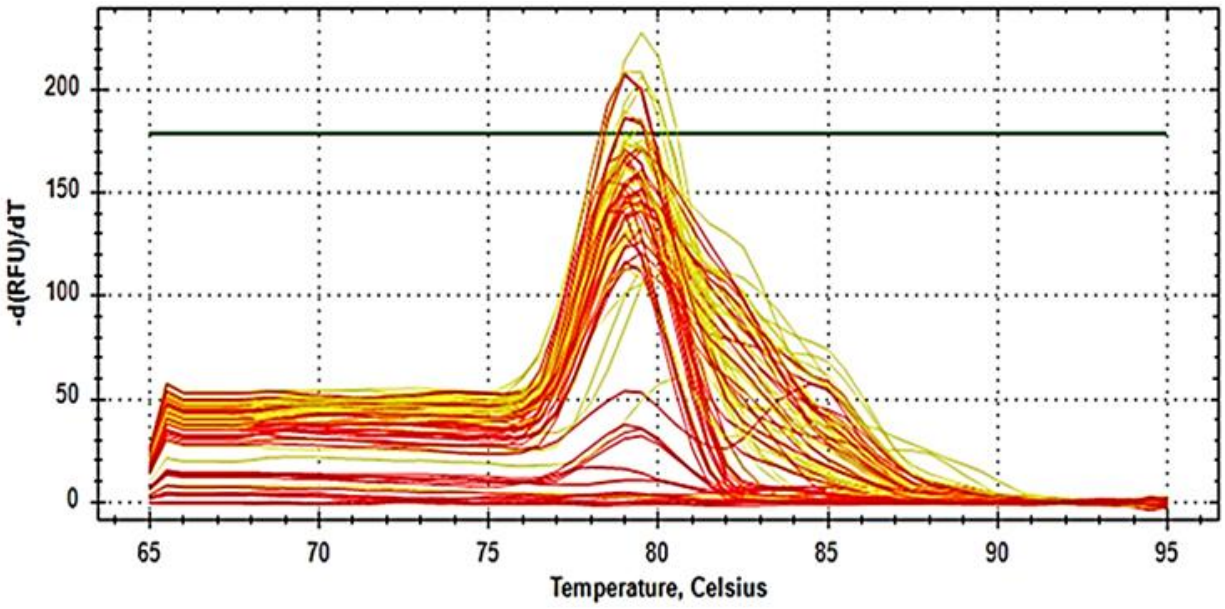

Fig. 4. Disassociation melting curve analysis with the samples tested for the gene of interest (ADIPOQ) in the β-TM patient group (G2, yellow line) and the control group (G1, red line)

Discussion

Table 1 presents the comparison of the mean± SD findings of the control group (G1) and β-TM patients (G2). The mean ± SD values at the P-value demonstrate the measurement rates for both healthy controls (G1) and β-TM patients (G2). As shown in Table 1, the analysis revealed significantly different mean values of several parameters (blood urea, total bilirubin, total protein, HCT, HGB, PLT, RBCs, and WBCs) and the ELISA measurements of MDA and SOD between the first group (G1), which represented healthy individuals, and the second group (G2), which consisted of beta-thalassemia major patients. The P-values in the β-TM patient group (G2) were significantly lower than those in the control group (G1) ($P < 0.05$).

A higher glomerular filtration rate (GFR) and reduced muscle mass are often the causes of decreased serum creatinine levels. Because creatinine is a consequence of muscle metabolism, chronic anemia causes poor development and muscle atrophy, particularly in individuals who are untreated or have had subpar transfusions. This lowers creatinine production (Al-Dagestani et al., 2025). A hyperdynamic circulatory condition brought on by chronic anemia also raises renal perfusion and GFR, which improves creatinine clearance. Frequent transfusion-induced iron excess may potentially change the renal function and impact creatinine levels. Therefore, low creatinine in these individuals indicates both improved renal clearance and decreased generation (Romadhon et al., 2024).

Prolonged kidney damage from various factors is the main cause of elevated blood urea nitrogen (BUN) levels. Frequent blood transfusions result in systemic iron overload, and excessive iron accumulation in renal tubular cells leads to tubular dysfunction and oxidative stress (Sadeghi et al., 2021). Renal hypoxia induced by chronic anemia promotes tubular and glomerular damage. Further contributing to progressive nephropathy are compensatory mechanisms such as renal hyperperfusion and hyperfiltration. Blood urea levels increase due to decreased urea clearance resulting from these renal abnormalities. Additionally, infections and dehydration—both common in these individuals—may exacerbate azotemia (Arian et al., 2025).

Chronic intramedullary and extravascular hemolysis is the main cause of elevated total bilirubin. While circulating aberrant red blood cells are removed from the reticuloendothelial system and spleen, ineffective erythropoiesis results in the premature loss of erythroid precursors in the bone marrow. Large quantities of heme are released during this severe hemolysis, and this heme is converted to unconjugated (indirect) bilirubin. Although the liver conjugates bilirubin, hyperbilirubinemia often results from high bilirubin loads that surpass the hepatic clearance capacity. Iron excess and repeated transfusions may also affect the liver function, lowering bilirubin conjugation and excretion and raising total bilirubin levels.

Reduced amounts of total protein may be caused by a number of conditions, including inefficient erythropoiesis and chronic anemia, which raise metabolic demands and result in protein-energy malnutrition, particularly in children. Hepatic protein production, especially albumin, may also be hampered by liver dysfunction induced by iron excess from constant transfusions (Akbarnejad et al., 2022). If iron-induced renal damage results in proteinuria, protein loss may also happen via the kidneys. Chronic inflammation also changes protein metabolism and may lead to an increase in serum protein catabolism in thalassemia. Affected patients have lower total protein levels as a result of malnutrition, liver disease, renal protein loss, and impaired protein turnover (Ahmadi et al., 2024).

Hemoglobin synthesis is substantially hampered by either significantly reduced beta-globin chain formation or its absence, resulting in a lowered hematocrit (HCT). An overabundance of unpaired alpha-globin chains arises due to this, precipitating within erythroid precursors and leading to oxidative damage and marrow death, a process called inefficient erythropoiesis (Lal et al., 2021). The production of red blood cells becomes significantly reduced because the majority of immature cells die before maturing. Abnormal circulating erythrocytes also experience hemolysis, or early destruction, which further reduces the quantity of red blood cells and dramatically lowers HCT (Gupta, 2024).

A genetic abnormality leads to a significant decrease or an absence of the the production of beta-globin chains, which are necessary components of adult hemoglobin (HbA), thereby lowering the hemoglobin levels. Unpaired alpha chains build up in erythroid precursors due to the imbalance between alpha and beta-globin chains, resulting in oxidative damage and early death of bone marrow cells (ineffective erythropoiesis) (Nahi & Jabir, 2023). The few red blood cells that enter circulation are also abnormal and susceptible to hemolysis, or premature destruction. Severe, chronic anemia is the consequence of inefficient erythropoiesis and excessive hemolysis, which together substantially decrease the hemoglobin concentration (Coates, 2024).

The main cause of thrombocytosis, or elevated platelet count, is splenectomy, which is often conducted to lessen hemolysis and enhance anemia. Increased circulating platelets result from the spleen's natural function of sequestering and eliminating extra platelets (Akbayram & Örkmez, 2020). The platelet production is further increased by compensatory bone marrow hyperplasia, which includes megakaryocyte proliferation, induced by prolonged anemia and inefficient erythropoiesis. The marrow activity may also be stimulated by chronic inflammation and iron excess (Mahesar et al., 2020).

Peripheral hemolysis combined with inadequate erythropoiesis causes a decline in the red blood cell (RBC) count. Normal hemoglobin (HbA) generation is disrupted by mutations in the HBB gene, leading to significant decrease or vanishing of beta-globin chain synthesis (Tzounakas et al., 2021). As a result, there are too many unpaired alpha-globin chains, which precipitate in erythroid precursors and cause oxidative damage and bone marrow death. The production of RBCs is significantly decreased as a consequence of the majority of erythroid cells dying before developing. Reduced RBC counts also result from the structural abnormalities and early destruction of the RBCs that enter circulation, particularly in the spleen (Islam et al., 2021).

Several conditions may cause elevated numbers of white blood cells (WBCs), including frequent blood transfusions and chronic anemia that stimulates the bone marrow and causes compensatory hyperplasia, which may slightly increase WBC production. Splenectomy, which is often performed to mitigate hemolysis, also plays a big role. Since the spleen typically filters and eliminates excessive or old WBCs, its absence might result in chronic leukocytosis. Moreover, recurring infections due to immunological dysfunction or iron excess may cause inflammatory reactions and boost WBC counts even further. Therefore, marrow activity, a decrease in splenic clearance, or underlying infections might be the causes of elevated WBCs in such individuals (Khawaji et al., 2020).

Increased oxidative stress elevates the levels of malondialdehyde (MDA). Reactive oxygen species (ROS) are produced when too many unpaired alpha-globin chains precipitate in red blood cells. Lipid peroxidation causes damage to cellular membranes. This lipid peroxidation process produces MDA, a by-product that acts as a biomarker for oxidative damage (Shakir & Al-Husseini, 2021). Generation of ROS is further increased by Fenton reactions, which are catalyzed by chronic iron excess due to repeated blood transfusions. Patients with beta thalassemia major have high MDA levels, which represent continuous cellular and membrane damage, as a consequence of iron-induced oxidative injury, inefficient erythropoiesis, and diminished antioxidant defenses (Al-Dhalimi & Al-Abady, 2025).

Chronic oxidative stress triggers a compensatory response that results in elevated levels of superoxide dismutase (SOD). Excess reactive oxygen species (ROS), particularly superoxide anions, are produced by the disease's hallmarks of inefficient erythropoiesis and persistent hemolysis. Erythroid precursors and mature red blood cells contain unpaired alpha-globin chains that precipitate, resulting in oxidative damage and membrane damage (Inteek & Madhkhoor, 2024). Additionally, chronic blood transfusions cause systemic iron excess, which triggers Fenton reactions to produce ROS. Superoxide dismutase, which transforms superoxide radicals into hydrogen peroxide, is one of the antioxidant defenses that the body upregulates in response. Thus, continuous adaptation to oxidative stress is reflected in elevated SOD (Arsalan et al., 2020).

In Table 2, the mean ± SD findings for the control (G1) and β-TM (G2) are compared. The mean ± SD values at the designated P-value represent the measurement rates for the β-TM patients (G2) and the healthy individuals (G1). The results of the study revealed that the first group (G1), which consisted of healthy controls, and the second group (G2), the β-TM patients, had different mean values of gene expression of adiponectin (Table 2). In the group of β-TM patients (G2), the P-values were significantly lower than those in the control group (G1) ($P < 0.05$).

Figure 1 displays the amplification curve that shows the expression levels of the housekeeping gene GAPDH in two groups: healthy control subjects (G1, blue lines) and patients with β-thalassemia major (β-TM) (G2, red lines). Higher gene expression is indicated by lower Ct values, which are inversely correlated with the quantity of starting template in quantitative PCR (qPCR). The cycle threshold (Ct) is the point at which the amplification curve crosses the threshold line, usually about 200 RFU. In this image, the red curves representing the β-TM group cross the threshold prior to the blue curves of the control group, indicating that the patients with β-TM exhibited higher levels of GAPDH expression. Since GAPDH is involved in glycolysis and is often elevated in such circumstances, this discovery could be attributed to increased metabolic activity or cellular stress in the β-TM samples. Overall, the findings show a significant difference in the GAPDH expression between the two groups, which may be significant for normalization in gene expression studies or as a possible indicator of altered cellular function in patients with β-TM.

Figure 2 illustrates the melting behavior of the GAPDH gene amplicons in the β-thalassemia major (β-TM) patients (represented by G2, red lines) and the control subjects (represented by G1, blue lines) in the dissociation (melting) curve analysis above. To determine the specificity of the PCR amplification, this graph shows the negative derivative of fluorescence with respect to temperature (-d(RFU)/dT) plotted against temperature (°C). The observation of a single, distinct peak at 82–84 °C in both groups suggests that a single PCR product was amplified in every sample. The absence of extra peaks confirms that primer-dimers and nonspecific products were not present. Both the patient and control samples seem to have amplified GAPDH fragments of the same size and sequence, as shown by the tight alignment of melt peaks between the red and blue curves. Since nonspecific amplification artifacts are not the cause of the observed variations in gene expression, this melt curve analysis supports the accuracy of the amplification data.

The expression of the ADIPOQ gene in the β-thalassemia major (β-TM) patients (represented by G2, yellow lines) is contrasted with that of the healthy control subjects (represented by G1, green lines) in the amplification curve above. The relative expression level of the gene is reflected in the cycle threshold (Ct) of quantitative PCR (qPCR), which is the point at which the amplification curve exceeds the predetermined threshold. Greater Ct values indicate lower expression, while lower Ct values indicate greater expression (Hasoon et al., 2020). In Figure 3, the yellow curves (the β-TM patients) often cross the threshold line between 28 and 35 cycles, whereas the green curves (the control) typically cross it earlier (about 22 to 28 cycles). This suggests that the β-TM patients had lower levels of ADIPOQ expression than the control subjects. While the yellow group's delayed amplification indicates lower gene expression, the green group's increasing trend indicates a more plentiful beginning template (Abd et al., 2022). The adiponectin gene regulates lipid metabolism and adipocyte signaling, both of which may be disrupted in β-TM patients, as seen by the downregulation of ADIPOQ. Understanding the metabolic or inflammatory changes linked to β-thalassemia may be aided by these results (Sadiq & Hasan, 2024).

The study of the dissociation (melting) curve demonstrates the specificity of the ADIPOQ gene amplification in both healthy individuals (G1, red lines) and patients with β-thalassemia major (β-TM) (G2, yellow lines). The peak on a melt curve indicates the temperature at which the double-stranded DNA product denatures, or melts, and its location and sharpness reveal the amplified product's consistency and specificity. Both the yellow (β-TM) and red (control) samples in Figure 4 show a single, clear peak between 80 and 83 °C, indicating that the PCR amplified a particular and consistent product in every sample (Caprari et al., 2023). A lack of nonspecific amplification or primer-dimer production is shown by the absence of extra peaks or shoulders, which strengthens the amplification data's dependability. The presence of a single, distinct melting peak overall indicates that the observed gene expression differences in the amplification plot are biologically relevant and not the result of technical artifacts (Mustafa et al., 2022). However, the slight variation in peak height and width between the groups may be due to minor heterogeneity in the melting behavior or variations in product concentration. According to this study, the downregulation of ADIPOQ in β-TM patients, as observed in the amplification curve, is supported by precise and targeted qPCR amplification (Abdulhaleem et al., 2025).

Conclusion

The study concluded that there was a decrease in the level of expression of the adiponectin gene. The study also found an increase in the levels of the oxidant stress marker (MDA), which caused an increase in the level of the antioxidant stress marker (SOD) as a compensatory response to reduce the damage of oxidant stress. Therefore,

these parameters are diagnostic or monitoring tools supporting the diagnosis of β-TM disease.

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