Journal of Kufa for Chemical Sciences
ISSN(P) :2077-2351 ISSN(E): 3006-3280
Journal Homepage:
https://journal.uokufa.edu.iq/index.php/jkcs/index

Article

Interleukin-1β and Glycolytic Enzyme Alterations in Beta-Thalassemia Major: Insights into Inflammation-Metabolism Crosstalk

Karima Jalil Hamza[1]* and Maysoon Khalid Hussein[2]

[1,2] Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq

*Corresponding author: kareema.hamza2305@sc.uobaghdad.edu.iq

ABSTRACT

Background: Beta-thalassemia major is characterized by chronic hemolytic anemia, ineffective erythropoiesis, and systemic iron overload, leading to metabolic and inflammatory alterations. **Objective:** This study investigated the relationship between serum interleukin-1β (IL-1β), key glycolytic enzymes (hexokinase [HK], pyruvate kinase [PK], phosphofructokinase [PFK]), and PFKP gene expression in beta-thalassemia major patients. **Methods:** A case-control study was conducted involving 42 beta-thalassemia major patients and 30 healthy controls. Serum levels of IL-1β, HK, PK, and PFK were measured using ELISA, while PFKP gene expression was assessed by RT-qPCR. **Results:** Patients exhibited significantly elevated IL-1β (41.34 ± 42.23 vs. 21.43 ± 26.45 pg/mL; $p = 0.0276$), HK (327.71 ± 282.40 vs. 131.47 ± 158.67 pg/mL; $p = 0.0012$), and PFK (1.83 ± 1.52 vs. 0.51 ± 0.53 ng/mL; $p < 0.0001$) compared to controls. Strong positive correlations were observed between IL-1β and glycolytic enzymes ($r = 0.637$-0.690, $p < 0.0001$). **Conclusion:** These findings demonstrate significant inflammation-metabolism crosstalk in

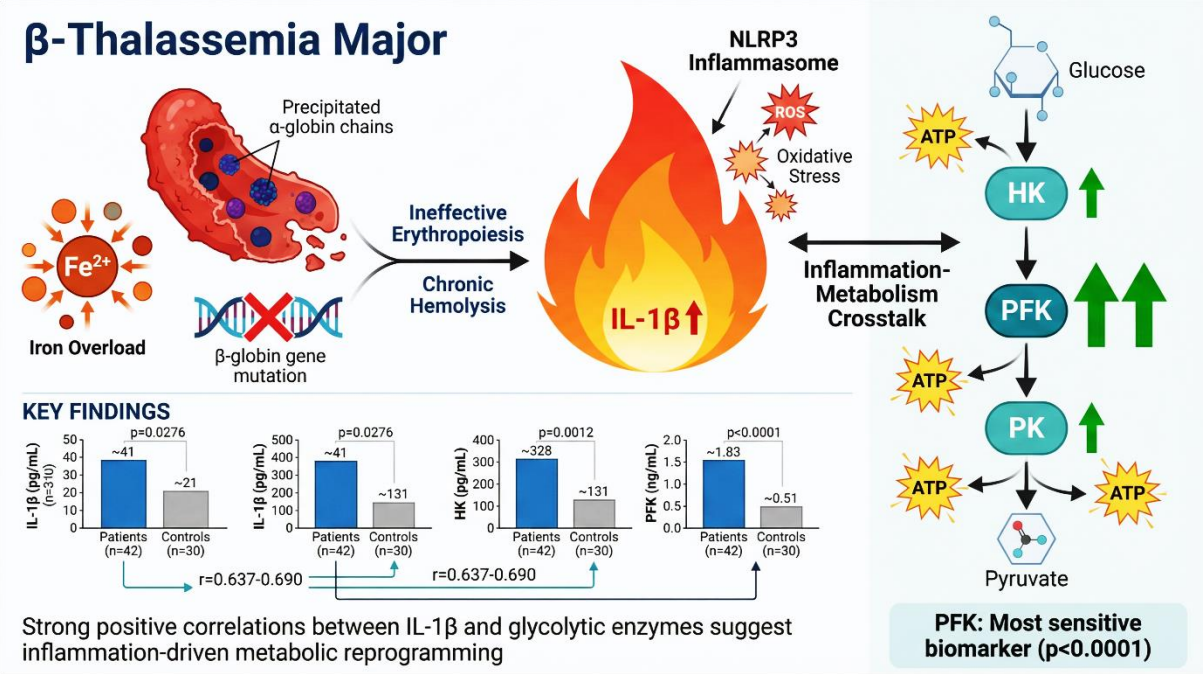
beta-thalassemia major, suggesting that targeting inflammatory pathways may ameliorate metabolic disturbances in affected patients.

HIGHLIGHTS

- IL-1β levels are significantly elevated in beta-thalassemia major patients, indicating chronic inflammation.
- Glycolytic enzymes (HK, PFK) are markedly increased, reflecting metabolic adaptation to oxidative stress.
- Strong positive correlations exist between IL-1β and all glycolytic enzymes measured.
- PFK demonstrates the highest diagnostic value as a biomarker ($p < 0.0001$).
- Inflammation-driven metabolic reprogramming may represent a therapeutic target in thalassemia.

Keywords: Beta-thalassemia major; Interleukin-1β; Glycolytic enzymes; Hexokinase; Pyruvate kinase; Phosphofructokinase; PFKP gene expression; Inflammation; Metabolic alteration

GRAPHICAL ABSTRACT:



1. INTRODUCTION

Beta-thalassemia major represents one of the most prevalent and clinically significant monogenic disorders worldwide, characterized by severely reduced or absent synthesis of beta-globin chains. This imbalance leads to precipitation of excess alpha-globin chains, ineffective erythropoiesis, chronic hemolytic anemia, and progressive iron overload, collectively contributing to multiorgan dysfunction and reduced quality of life [1,2]. In Iraq, beta-thalassemia constitutes a major public health burden, with carrier rates of approximately 3.5% and high consanguinity rates exceeding 57%, contributing to elevated disease prevalence [3–6]. The pathophysiology of beta-thalassemia extends beyond simple hemoglobin deficiency to encompass a complex interplay of oxidative stress, chronic inflammation, and metabolic dysregulation. Ineffective erythropoiesis and iron overload generate reactive oxygen species (ROS) through Fenton chemistry, overwhelming cellular antioxidant defenses and causing membrane damage, protein oxidation, and lipid peroxidation [7,8]. This oxidative environment activates inflammatory pathways, particularly the NLRP3 inflammasome, leading to sustained production of pro-inflammatory cytokines including interleukin-1β (IL-1β) [9]. Mature erythrocytes, lacking mitochondria, depend entirely on glycolysis for ATP production. The key regulatory enzymes—hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK)—control glucose metabolism and are critical for maintaining red blood cell integrity [10,11]. Recent evidence suggests that inflammatory cytokines can modulate glycolytic enzyme expression through metabolic reprogramming, a phenomenon resembling the Warburg effect observed in activated immune cells [12,13]. However, the specific relationship between IL-1β and glycolytic enzyme alterations in beta-thalassemia remains inadequately characterized. This study aimed to investigate the interrelationship between inflammatory markers (IL-1β) and glycolytic enzymes (HK, PK, PFK) in Iraqi patients with beta-thalassemia major, alongside assessment of PFKP gene expression. Understanding this inflammation-metabolism axis may identify potential biomarkers and therapeutic targets for improved disease management.

2. MATERIALS AND METHODS

2.1. Study Design and Participants

A case-control study was conducted at the Center for Genetic Blood Diseases (Thalassemia Center), Al-Diwaniyah Health Directorate, Iraq, from October 2024 to February 2025. The study included 42 patients diagnosed with beta-thalassemia major and 30 age-matched healthy controls (age range: 6-35 years). Diagnosis was confirmed by hemoglobin electrophoresis showing elevated HbF and reduced/absent HbA, according to Thalassemia International Federation guidelines. Patients with other thalassemia types, systemic diseases (diabetes, liver failure, heart failure), chronic infections, malignancies, or acute illness at sampling were excluded. The study was approved by the institutional ethics committee, and informed consent was obtained from all participants.

2.2. Sample Collection

Six milliliters of venous blood was collected from each participant between 8:00 AM and 2:00 PM. Blood samples from thalassemia patients were obtained before transfusion. Two milliliters were collected in K_3-EDTA tubes for RNA extraction, while four milliliters were placed in gel tubes, allowed to clot for 15 minutes at room temperature, and centrifuged at 3600 rpm for 6 minutes. Separated serum was aliquoted and stored at -20°C until biochemical analysis.

2.3. Biochemical Analysis

Serum levels of IL-1β (Elabscience®, USA), hexokinase (Bioassay, China), pyruvate kinase (Bioassay, China), and phosphofructokinase (Bioassay, China) were quantified using commercial sandwich ELISA kits following manufacturers' protocols. Briefly, 40-50 µL of samples or standards were added to pre-coated microplate wells with biotinylated detection antibody and HRP conjugate. After incubation (60 min, 37°C) and washing (5×), TMB substrate was added and incubated (10 min, 37°C, dark). Stop solution was added, and optical density was measured at 450 nm within 10 minutes. Concentrations were calculated from standard curves (R^2 values: IL-1β = 0.975, HK = 0.970, PK = 0.990, PFK = 0.995).

2.4. RNA Extraction and cDNA Synthesis

Total RNA was extracted from blood samples using TRIzol™ reagent (Invitrogen, USA) according to manufacturer's instructions. RNA concentration was quantified using Qubit 4 Fluorometer (Invitrogen). Complementary DNA (cDNA) was synthesized using EasyScript® First-Strand cDNA Synthesis SuperMix (Transgen, China) with the following thermocycler conditions: priming at 25°C for 10 min, reverse transcription at 50°C for 60 min, enzyme inactivation at 80°C for 5 min, and storage at 12°C.

2.5. Quantitative Real-Time PCR (RT-qPCR)

PFKP gene expression was analyzed using RT-qPCR (Bioer LineGene 4800, Japan) with GAPDH as the internal reference gene. Primers were designed using NCBI Primer-BLAST: PFKP-F: 5'-GACTCAGGATGTGCAGAAGGC-3', PFKP-R: 5'-CCCCACGTTGATGACAGCTAC-3' (169 bp); GAPDH-F: 5'-ATCACCATCTTCCAGGAGCGA-3', GAPDH-R: 5'-CAGAGGGGGCAGAGATGATGA-3' (157 bp). Reactions (20 µL) contained 10 µL Luna Universal qPCR Master Mix, 1 µL each primer (10 µM), 5 µL cDNA, and 3 µL nuclease-free water. Thermocycling conditions: initial denaturation (95°C, 5 min); 40 cycles of denaturation (95°C, 20 sec), annealing (56.3°C, 30 sec), and extension (72°C, 30 sec); followed by melting curve analysis. Relative expression was calculated using the $2^{-}\Delta\Delta Ct$ method.

Table 1: Primer sequences used for RT-qPCR analysis

Gene	Sequence (5'-3')	Product Size
PFKP-F	GACTCAGGATGTGCAGAAGGC	169 bp
PFKP-R	CCCCACGTTGATGACAGCTAC	
GAPDH-F	ATCACCATCTTCCAGGAGCGA	157 bp
GAPDH-R	CAGAGGGGGCAGAGATGATGA	

2.6. Statistical Analysis

Data were analyzed using SPSS version 26 and Microsoft Excel 2016. Results are expressed as mean ± standard deviation (SD). Independent

sample t-test was used for between-group comparisons. Pearson correlation coefficient was used to assess relationships between variables. Statistical significance was set at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Serum IL-1β Levels

Serum IL-1β levels were significantly elevated in beta-thalassemia patients (41.34 ± 42.23 pg/mL) compared to healthy controls (21.43 ± 26.45 pg/mL; $p = 0.0276$), as shown in Table 2 and Figure 1. The range was notably wider in patients (3.81-140.75 pg/mL) than controls (3.44-61.07 pg/mL), indicating greater variability in inflammatory status among thalassemia patients. These findings align with previous reports demonstrating chronic inflammation in beta-thalassemia. The elevated IL-1β likely results from multiple factors: iron-mediated NLRP3 inflammasome activation, oxidative stress-induced inflammatory signaling, and macrophage activation from continuous phagocytosis of damaged erythrocytes [14,15]. Iron overload, common in transfusion-dependent patients, catalyzes ROS formation through Fenton chemistry, which activates inflammasome components and promotes IL-1β maturation [16,17]. Furthermore, IL-1β suppresses erythropoietin production and inhibits erythroid progenitor proliferation, potentially exacerbating the anemia of chronic inflammation [18,19].

Table 2: Comparison of serum IL-1β levels between groups

Parameter	Control (n=30)	Patients (n=42)	p-value
IL-1β (pg/mL)	21.43 ± 26.45	41.34 ± 42.23	**0.0276***
Range	3.44 - 61.07	3.81 - 140.75	

**Significant at $p \leq 0.05$; Data expressed as Mean ± SD*

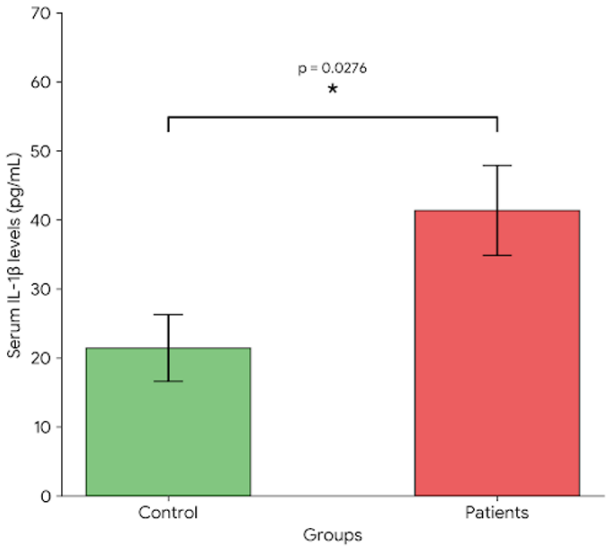


Figure 1: Serum IL-1β levels in beta-thalassemia patients and controls. Data expressed as mean ± SEM. *p ≤ 0.05

3.2. Serum Glycolytic Enzyme Levels

All three glycolytic enzymes showed elevated levels in beta-thalassemia patients compared to controls (Table 3; Figures 2). Hexokinase (HK) was significantly elevated (327.71 ± 282.40 vs. 131.47 ± 158.67 pg/mL; p = 0.0012). Phosphofructokinase (PFK) demonstrated the most statistically significant elevation (1.83 ± 1.52 vs. 0.51 ± 0.53 ng/mL; p < 0.0001). Pyruvate kinase (PK) showed a trend toward elevation (227.00 ± 288.48 vs. 116.30 ± 158.38 pg/mL; p = 0.0643) that did not reach statistical significance.

Table 3: Comparison of serum glycolytic enzyme levels between groups

Parameter	Control (n=30)	Patients (n=42)	p-value
HK (pg/mL)	131.47 ± 158.67	327.71 ± 282.40	**0.0012****
PK (pg/mL)	116.30 ± 158.38	227.00 ± 288.48	0.0643
PFK (ng/mL)	0.51 ± 0.53	1.83 ± 1.52	**<0.0001****

***p ≤ 0.01; ****p ≤ 0.0001; Data expressed as Mean ± SD*

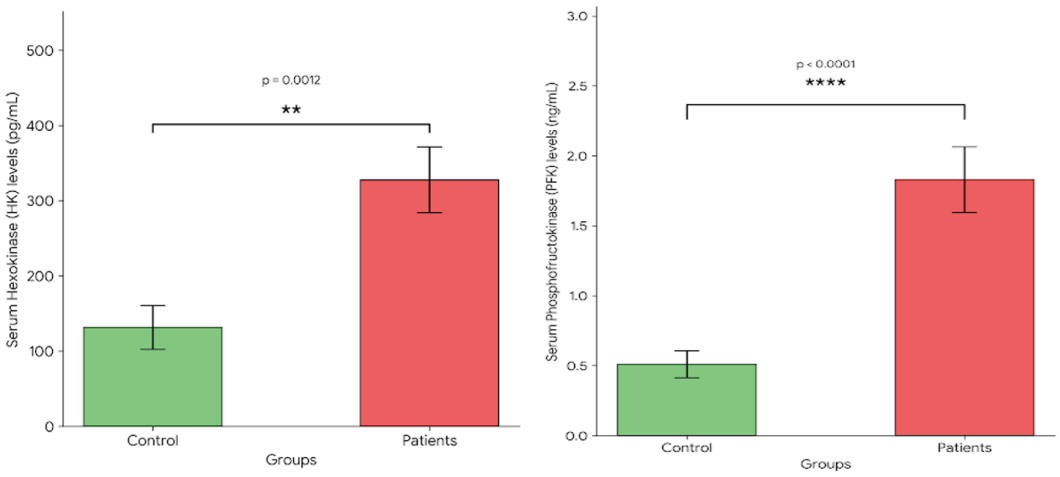


Figure 2: Serum HK (left) and PFK (right) levels. **p ≤ 0.01, ****p ≤ 0.0001 vs. control

The elevated glycolytic enzyme levels in beta-thalassemia patients may reflect multiple mechanisms: (1) increased glycolytic activity as a compensatory response to chronic hemolysis; (2) release from damaged or lysed erythrocytes; (3) hypoxia-driven upregulation through HIF-1α signaling; and (4) inflammatory cytokine-mediated metabolic reprogramming [20,21]. Young erythrocytes and reticulocytes, which are increased in thalassemia due to accelerated erythropoiesis, possess higher glycolytic enzyme activities than mature red cells [22]. Additionally, the particularly marked elevation of PFK ($p < 0.0001$) suggests this enzyme may serve as a sensitive biomarker of metabolic stress in beta-thalassemia.

3.3. PFKP Gene Expression

RT-qPCR analysis revealed variable PFKP gene expression patterns (Table 4; Figure 3). Mean fold change was higher in patients (51.01 ± 145.76) compared to controls (16.49 ± 43.36), though this difference did not reach statistical significance ($p = 0.219$). The high standard deviations reflect substantial inter-individual variability in both groups.

Table 4: PFKP gene expression analysis by RT-qPCR

Parameter	Control (n=30)	Patients (n=42)	p-value
PFKP Ct	25.80 ± 1.43	27.39 ± 2.08	-
GAPDH Ct	22.00 ± 3.28	23.27 ± 4.47	-
Fold Change	16.49 ± 43.36	51.01 ± 145.76	0.219

Ct: Cycle threshold; Fold Change calculated using $2^{-\Delta\Delta Ct}$ method

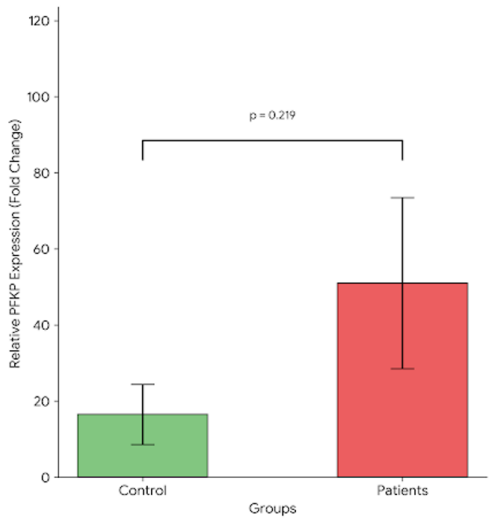


Figure 3: PFKP gene expression (Fold Change) in patients and controls. Data expressed as mean ± SEM

The discordance between elevated serum PFK protein and non-significant PFKP gene expression changes suggests that post-transcriptional mechanisms predominantly regulate serum enzyme levels in beta-thalassemia. These may include protein stability, enzyme release during hemolysis, and clearance rates. Furthermore, peripheral blood mRNA may not accurately represent expression in erythroid precursors or reticulocytes, the cells most relevant to the disease pathophysiology [23,24].

3.4. Correlation Analysis

Pearson correlation analysis revealed significant positive associations between IL-1β and all glycolytic enzymes: IL-1β vs. HK ($r = 0.657$, $p < 0.0001$), IL-1β vs. PK ($r = 0.690$, $p < 0.0001$), and IL-1β vs. PFK ($r = 0.637$, $p < 0.0001$) (Table 5; Figures 4). Additionally, strong correlations were observed among glycolytic enzymes, with the strongest between HK and PFK ($r = 0.874$, $p < 0.0001$). PFKP gene expression did not correlate significantly with any parameter.

Table 5: Pearson correlation matrix between studied parameters in beta-thalassemia patients

Parameter	IL-1β	HK	PK	PFK	PFKP FC
IL-1β	1.000	**0.657*** **	**0.690*** **	**0.637*** **	-0.161
HK		1.000	**0.509*** **	**0.874*** **	-0.088

PK	1.000	**0.558*** **	-0.162
PFK		1.000	0.006

****p < 0.001; FC: Fold Change*

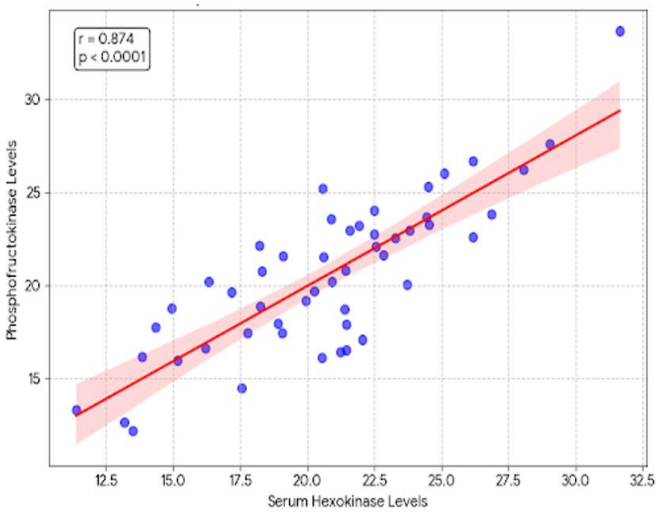


Figure 4: Scatter plot showing the correlation between serum HK and PFK levels (r = 0.874, p < 0.0001)

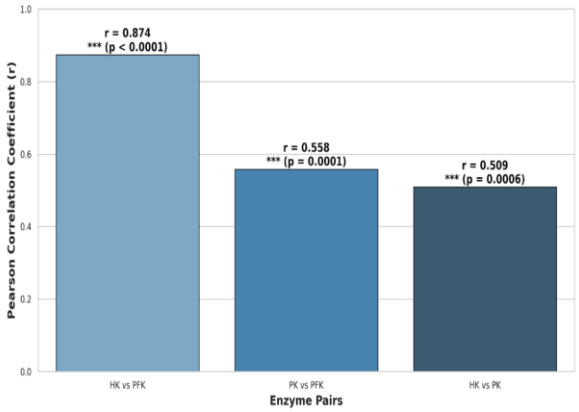


Figure 5: Correlation heatmap showing relationships between all studied parameters. Color intensity represents correlation strength. ***p < 0.001

These significant correlations between IL-1β and glycolytic enzymes support the concept of inflammation-driven metabolic reprogramming in beta-thalassemia. Pro-inflammatory cytokines induce metabolic shifts toward glycolysis through NF-κB and HIF-1α signaling, similar to the Warburg effect observed in activated immune cells [25,26]. The very strong correlation between HK and PFK (r = 0.874) suggests coordinated regulation of these rate-limiting enzymes, likely reflecting both increased glycolytic capacity and their common release during hemolysis. These

findings indicate that targeting inflammatory pathways may have secondary benefits on metabolic disturbances in beta-thalassemia.

3.5. Summary of Findings

The present study demonstrates that beta-thalassemia major is associated with significant inflammatory and metabolic alterations. Serum IL-1β levels were significantly elevated in patients, confirming the presence of chronic inflammation. In parallel, key glycolytic enzymes, particularly hexokinase and phosphofructokinase, were markedly increased, indicating enhanced glycolytic activity and metabolic adaptation to chronic hemolysis and oxidative stress. Phosphofructokinase showed the strongest statistical significance, suggesting its potential value as a sensitive metabolic biomarker. In contrast, pyruvate kinase exhibited a non-significant increase, reflecting variable enzymatic regulation among patients. Although serum PFK levels were elevated, PFKP gene expression did not differ significantly between groups, implying predominant post-transcriptional regulation rather than transcriptional upregulation. Collectively, these findings support a strong inflammation–metabolism crosstalk in beta-thalassemia major, with IL-1β likely contributing to glycolytic reprogramming.

Table 6: Summary of all studied parameters

Parameter	Control (n=30)	Patients (n=42)	p-value	Sig.
IL-1β (pg/mL)	21.43 ± 26.45	41.34 ± 42.23	0.0276	*
HK (pg/mL)	131.47 ± 158.67	327.71 ± 282.40	0.0012	**
PK (pg/mL)	116.30 ± 158.38	227.00 ± 288.48	0.0643	NS
PFK (ng/mL)	0.51 ± 0.53	1.83 ± 1.52	<0.0001	****
PFKP FC	16.49 ± 43.36	51.01 ± 145.76	0.219	NS

**p<0.05, **p<0.01, ****p<0.0001; NS: Non-significant; FC: Fold Change; Data as Mean ± SD*

4. CONCLUSION

This study demonstrates significant inflammatory and metabolic alterations in Iraqi patients with beta-thalassemia major. The key findings include: (1) significantly elevated serum IL-1β levels, confirming chronic inflammation in this condition; (2) markedly increased glycolytic

enzymes, particularly PFK, reflecting metabolic adaptation and/or hemolysis-related enzyme release; (3) strong positive correlations between IL-1β and all glycolytic enzymes, supporting inflammation-metabolism crosstalk; and (4) discordance between PFKP gene expression and serum protein levels, suggesting predominant post-transcriptional regulation. These findings have important clinical implications. PFK may serve as a sensitive biomarker for disease monitoring, given its highly significant elevation. The strong correlations between inflammatory and metabolic markers suggest that anti-inflammatory therapies, such as IL-1β receptor antagonists, might have beneficial effects on metabolic disturbances in beta-thalassemia. Furthermore, the emerging pyruvate kinase activators represent a promising therapeutic strategy for improving erythrocyte metabolism and reducing hemolysis.

REFERENCES

[1] Taher AT, Weatherall DJ, & Cappellini MD. [2018]: Thalassaemia. Lancet, 391(10116), pp. 155–167.

[2] Kattamis A, Forni GL, Aydinok Y, & Viprakasit V. [2020]: Changing patterns in the epidemiology of β-thalassemia. Eur. J. Haematol., 105(6), pp. 692–703.

[3] Al-Allawi NA, & Al-Dousky AA. [2010]: Frequency of haemoglobinopathies at premarital health screening in Duhok, Iraq. J. Med. Screen., 17, pp. 124–127.

[4] AL-SARRAY A, El-YASSIN H, & JAWAD A. [2023]: The Prime Mutation of β-Thalassemia and Disease Severity in (Wasit and Maysan) South of Iraq. J. Nat. Sc. Biol. Med., 14, pp. 221–224.

[5] Hamed OM, Al-Taii RA, & Jankeer MH. [2021]: Biochemical and Genetic Study in Blood of β-Thalassaemia Children in Mosul City, Iraq. Iraqi Journal of Science, 62, pp. 2501–2508.

[6] Al-Husseiny IA, & Al-Jumaili EF. [2025]: Comparative Analysis of the Age, Gender, and Ferritin Levels with Hepcidin in Iraqi Patients Suffering Beta Thalassemia (Major and Intermedia). HAYATI J. Biosci., 32, pp. 1518–1528.

[7] Rivella S. [2019]: Iron metabolism under conditions of ineffective erythropoiesis in β-thalassemia. Blood, 133(1), pp. 51–58.

[8] Vinchi F, Muckenthaler MU, Da Silva MC, et al. [2014]: Atherogenesis and iron: from epidemiology to cellular level. Front. Pharmacol., 5, Article 94.

[9] Swanson KV, Deng M, & Ting JP. [2019]: The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat. Rev. Immunol., 19, pp. 477–489.

[10] van Wijk R, & van Solinge WW. [2005]: The energy-less red blood cell is lost: erythrocyte enzyme abnormalities of glycolysis. Blood, 106(13), pp. 4034–4042.
[11] Grace RF, Zanella A, Neufeld EJ, et al. [2015]: Erythrocyte pyruvate kinase deficiency: 2015 status report. Am. J. Hematol., 90, pp. 825–830.
[12] Tannahill GM, Curtis AM, Adamik J, et al. [2013]: Succinate is an inflammatory signal that induces IL-1β through HIF-1α. Nature, 496, pp. 238–242.
[13] O'Neill LAJ, Kishton RJ, & Rathmell J. [2016]: A guide to immunometabolism for immunologists. Nat. Rev. Immunol., 16, pp. 553–565.
[14] Taher AT, Musallam KM, & Cappellini MD. [2021]: β-Thalassemias. N. Engl. J. Med., 384, pp. 727–743.
[15] Ganz T. [2013]: Systemic iron homeostasis. Physiol. Rev., 93, pp. 1721–1741.
[16] Muckenthaler MU, Rivella S, Hentze MW, & Galy B. [2017]: A red carpet for iron metabolism. Cell, 168, pp. 344–361.
[17] Majeed MS. [2017]: Evaluation of some Biochemical and Endocrine Profiles in transfusion-dependent Iraqi major β-thalassemia patients. Iraqi Journal of Science, 58, pp. 639–645.
[18] Weiss G, Ganz T, & Goodnough LT. [2019]: Anemia of inflammation. Blood, 133, pp. 40–50.
[19] Nemeth E, & Ganz T. [2023]: Hepcidin and iron in health and disease. Annu. Rev. Med., 74, pp. 261–277.
[20] Mor I, Cheung EC, & Vousden KH. [2011]: Control of glycolysis through regulation of PFK1: old friends and recent additions. Cold Spring Harb. Symp. Quant. Biol., 76, pp. 211–216.
[21] Semenza GL. [2010]: HIF-1: upstream and downstream of cancer metabolism. Curr. Opin. Genet. Dev., 20, pp. 51–56.
[22] Beutler E. [1979]: Red cell enzyme defects as nondiseases and as diseases. Blood, 54, pp. 1–7.
[23] Matte A, Federti E, Kung C, et al. [2021]: The pyruvate kinase activator mitapivat reduces hemolysis and improves anemia in a β-thalassemia mouse model. J. Clin. Invest., 131, Article e144206.
[24] Kuo KHM, Layton DM, Al-Samkari H, et al. [2024]: Mitapivat, a pyruvate kinase activator, in adults with alpha- or beta-thalassaemia: ENERGIZE, a phase 3, randomised, double-blind, placebo-controlled trial. Lancet, 403, pp. 155–167.
[25] Palsson-McDermott EM, & O'Neill LAJ. [2013]: The Warburg effect then and now: from cancer to inflammatory diseases. Bioessays, 35, pp. 965–973.
[26] Kelly B, & O'Neill LAJ. [2015]: Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Res., 25, pp. 771–784.