ISSN: 0067-2904

The Correlation of Erythrocytosis Familial 6 (ECYT6) Gene Expression and their Targeted miRNA in Patients with Beta-Thalassemia

Hiba M. A. Al-kafagi[1], Lubna F. Hussein[2], Maryam Q. Mohammed[3], Ghassan M. Sulaiman[1*]

[1] *Division of Biotechnology, Department of Applied Sciences, University of Technology, Baghdad, Iraq*
[2]*Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.*
[3]*Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq.*

Received: 27/9/2024 Accepted: 3/3/2025 Published: 30/3/2026

Abstract

Beta thalassemia is a genetic blood disorder that affects around 1.5% of the world's population. Beta globin, an essential component of hemoglobin, is involved in the development of this disease. The *HBB* gene, also known as the hemoglobin subunit beta gene, has been the focus of extensive studies, considering its crucial role in the production of hemoglobin. The associations between the *HBB* gene and various miRNA (miRNA) that serve as essential regulators of gene expression have been the subject of many investigations. In the current investigation, many blood indicators of thalassemia were assessed, RNA was extracted, cDNA was created, and quantitative real-time PCR was used to measure the expression of the *HBB* gene and *miR-361-3p*. The results showed a significant increase in the levels of ferritin and WBC in the patient group with a high significant difference ($P \leq 0.01$), while the HB value decreased in the patients with a high significant difference. Molecular results showed an increase in the gene expression level of the *HBB* gene with a value of (1.909) while a decrease in the gene expression level of the micro gene in thalassemia patients compared to the control group with a value of (0.692). The findings demonstrate that the downregulation of *miR-361-3p* results in an upregulation of its target genes, including *HBB*, leading to impaired erythropoiesis and abnormal red blood cell morphology, which are consistent with the pathophysiology of β-thalassemia. Consequently, it can be inferred that *miR-361-3p* plays a critical regulatory role in the pathogenesis of β-thalassemia by modulating *HBB* gene expression. The current study is limited by its small sample size, focus on a specific age group and single target gene. Future studies with a broader scope are recommended to overcome these limitations.

Keywords: β-thalassemia, Hemoglobin beta subunit (HBB) gene, *miR-361-3p*, Gene expression, complementary DNA.

العلاقة بين التعبير الجيني لجين كثرة كريات الدم الحمراء العائلية- ٦(ECYT6) و الحمض الريبوزي النووي الميكروي المستهدفة لديه في مرضى بيتا ثلاسيميا

هبة الخفاجي ، لبنى حسين ، مريم محمد ، غسان محمد سليمان

الخلاصه

*Email: ghassan.m.sulaiman@uotechnology.edu.iq

تعتبر بيتا ثلاسيميا أحد الأمراض الوراثية التي تصيب الدم، وتؤثر على حوالي 1.5٪ من سكان العالم. يلعب غلوبين بيتا، وهو مكون أساسي من الهيموغلوبين، دورًا مهمًا في تطور هذا المرض. لقد كان جين غلوبين بيتا، المعروف أيضًا باسم جين سلسلة بيتا للهيموغلوبين، محورًا للعديد من الدراسات نظرًا لدوره الحاسم في إنتاج الهيموغلوبين. تمثل الروابط بين جين غلوبين بيتا ومختلف جزيئات الحمض الريبوزي النووي الميكروي التي تعمل كمنظمات أساسية للتعبير الجيني موضوعًا للعديد من الاكتشافات. في هذه الدراسة الحالية، تم تقييم العديد من مؤشرات الدم لمرض الثلاسيميا، وتم استخراج الحمض النووي الريبوزي ، ثم بناء الحمض النووي الريبوزي منقوص الأوكسجين المتمم ، وبالتالي استخدام تقنية تفاعل البوليميراز المتسلسل الكمي في الوقت الحقيقي لقياس تعبير جين غلوبين بيتا (HBB) وجزيء (miR−361−3p). أظهرت النتائج زيادة كبيرة في مستويات الفيريتين والكريات البيضاء في مجموعة المرضى مع فرق كبير ذي دلالة إحصائية ($P\leq 0.01$)، في حين انخفضت قيمة الهيموغلوبين في المرضى مع فرق كبير ذي دلالة إحصائية. أظهرت نتائج الدراسة الجزيئية زيادة في مستوى تعبير جين غلوبين بيتا بقيمة (1.909) وانخفاضًا في مستوى تعبير جين الحمض الريبوزي النووي الميكروي في مرضى الثلاسيميا مقارنة بمجموعة الضوابط بقيمة (0.692). توضح النتائج أن انخفاض مستوى تعبير (miR−361−3p) يؤدي إلى زيادة تعبير الجينات المستهدفة، بما في ذلك (HBB)، مما يؤدي إلى ضعف تكوين كريات الدم الحمراء وتشوه شكلها، وهو ما يتوافق مع امراضية بيتا ثلاسيميا. وبالتالي، يمكن استنتاج أن التعبير الجيني للحمض الريبوزي النووي الميكروي يلعب دورًا تنظيميًا حاسمًا في حدوث بيتا ثلاسيميا من خلال تعديل تعبير جين غلوبين بيتا. تقتصر الدراسة الحالية على صغر حجم العينة والتركيز على فئة عمرية محددة وجين مستهدف واحد. يوصى بإجراء دراسات مستقبلية ذات نطاق أوسع للتغلب على هذه القيود.

1. Introduction

Beta thalassemia is the most severe form of inherited disorders. A genetic hemoglobin abnormality dubbed thalassemia is characterized by a decrease in red blood cell counts relative to normal and a low hemoglobin level [1]. Thalassemia is a potentially fatal hereditary anemia that can cause health problems if left untreated. The oxygen-carrying protein hemoglobin is reduced in thalassemia, which causes anemia symptoms like exhaustion and anxiety to develop [2]. In numerous regions of Iraq, the frequency of β-thalassemia (β-thal) carrier states varies from 3.7% to 4.6%, suggesting a very varied spectrum of mutations among the primarily Arab population in central Iraq. Numerous origins, including Mediterranean, Asian, Indian, Turkish, Kurdish, Iranian, Egyptian, and Saudi Arabian origins, are responsible for these alterations [3]. Hemoglobin (Hb) has a higher affinity for oxygen in familial erythrocytosis-6, which leads to compensatory polycythemia and reduced oxygen transport to peripheral organs. Because compensatory polycythemia ensures appropriate oxygen tissue delivery, patients are typically asymptomatic, and one of the prominent genes is hemoglobin subunit beta (HBB), which has been linked to the erythrocytosis familial-6 gene family. The decreases in detectable beta globin levels constitute a hallmark of beta-plus thalassemia. The National Center for Biotechnology Information (NCBI) describes the location of the *HBB* gene, which has been identified at 11p15.4, Exon count:3, as one of the erythrocytosis familial-6 (ECYT6) genes [4].

According to previous investigations carried out on the *HBB* gene, a vital function of this gene in the synthesis of hemoglobin (a protein that carries oxygen throughout the body) has been the subject of plenty of studies [5]. Important modulators of gene expression are miRNA. MiRNA is highly dysregulated in disease, even though it influences a broad spectrum of biological functions [6]. A class of small non-coding RNA molecules known as miRNAs is essential for controlling the expression of certain genes. Their primary mechanism of action involves binding to the 3' untranslated region (3' UTR) of target

messenger RNAs (mRNAs), which results in either mRNA destruction or translational suppression. Numerous cellular functions, including development, differentiation, and reaction to external stimuli, depend on this complex process; also, gene expression control by miRNA is a complex and multidimensional process; because of the imprecise nature of miRNA-mRNA binding, experimental validation is essential even when computational approaches may predict expected miRNA targets [7]. A complicated network of interactions can result from a single miRNA targeting many mRNAs and multiple miRNAs regulating a single mRNA, miRNA activity is dynamic and depends on environmental inputs, developmental stage, and cellular context. With their target genes, miRNAs can also create complex regulatory networks and feedback loops; the epigenetic changes affect miRNA expression and activity, making this process even more difficult. Also, a number of human diseases have been linked to dysregulation of miRNA expression and activity, highlighting the importance of comprehending these complexities in order to create effective therapeutic approaches[8].

In an attempt to effectively reduce the expression of target mRNAs, miRNA typically interacts with their 3′ untranslated region (3′ UTR), 5′ UTR coding sequence, or gene promoter [9]. Nonetheless, in some cases, they have been shown to act on certain genes and regulate transcription as well. Moreover, the control that miRNA exerts over their target genes is also complex and depends on a lot of factors like the localization of miRNA in the cell, the level of miRNA and target mRNAs, and the strength of the binding miRNA-target interactions[10]. The essences of beta-thalassemia are accountable to the existing miRNAs that control genes that are responsible for erythropoiesis, iron metabolism, and oxidative stress. Further investigations in this field can result in the development of novel therapies for this crippling disease which constitutes almost all major pediatric illnesses[11].

The miRNA has two ways to penetrate target cells: either through the proteins it binds to or via secretion into the extracellular fluid, from where they can be internalized by exosomes. The presence of extracellular miRNAs enables them to act as active participants in cell physiology and pathology and regulate cell-cell interactions [12]. MiRNA 361-3p is known to be an essential aforementioned factor for several cell biological activities such as cell proliferation, differentiation, and apoptosis [13, 14]. A wide range of studies have been conducted to explore the relationship Between *HBB* gene and various miRNAs like *miR-361-3p*. MicroRNAs, which are small and non-coding RNA molecules, are key components of gene expression regulation [15]. While older studies have hinted at the possibility of *miR-361-3p* having a role in hematopoietic cancers, this is the initial analysis done on its function on thalassemia. Studies have indicated that the other potential role of miR-361-3p could be in the initiation and or progression of certain hematological malignancies like multiple Myeloma Thalassemia which has the ability to diminish the cell's viability while increasing apoptosis or programmed cell death in lymphoma, which seems to be due to the Wnt/β-catenin signaling pathway which is essential for the pathogenesis of lymphomas [16, 17].

Restoring some miRNA levels or inhibiting its target genes could potentially improve erythropoiesis and iron metabolism, thereby alleviating the symptoms and complications of thalassemia. However, further research is needed to fully understand the role of miRNA in thalassemia and its potential therapeutic applications[18]. In considering the significant role that the *HBB* gene contributes in the onset and progression of thalassemia, besides the numerous mutations that have been found to affect this gene and its function, and in light of the most recent discovery of the critical function of some miRNA which has been shown to target specific genes and regulate gene expression a molecular marker for diagnosis in the

future. The present investigation tracked thalassemia patients; therefore, the objective was to evaluate the detrimental impact of *miR-361-3p's* gene expression on the *HBB* gene in Iraqi thalassemia patients by monitoring their gene expression.

2. Methods

Patients and Controls

The current investigation comprised one hundred participants as a case-control study from the Medical City Hospital in Baghdad between November 2021 and February 2022. Ethical approval was obtained from the University of Technology and Baghdad Health Directorate (Reference No. 340, Nov 25, 2022). Additional approvals were secured from the administration of Medical City Hospital in Baghdad, as well as private clinics and laboratories, with informed consent from each patient after explaining the study's purpose. 3 mL of whole blood collected through EDTA tubes. Obtaining a blood sample from thalassemia patients, getting a doctor to verify the diagnosis, and then keeping the blood sample in TRIZOL to prevent RNA degradation.

Inclusion Criteria

Patient group: participants must have a confirmed diagnosis of beta-thalassemia based on a medical report. Participants should not have any other known genetic disorders.
Healthy controls: Control participants should be in apparent good health and have no known blood disorders.

Exclusion Criteria

Patient group: Patients with alpha-thalassemia or other types of thalassemia should be excluded. Any participant with another blood disorder, whether hereditary or acquired, should be excluded. Healthy controls: should have no family history of thalassemia or other blood disorders

RNA Extraction

Using (TransZol Up Plus RNA Kit, TransGen Biotech company, China) with cat .no (ER501-01). Following the manufacturer's procedure, total RNA was directly extracted from the whole blood sample.

Concentration and Purity measurement

Using the (NANODROP ONE^{C}, Thermo Fisher Scientific, USA), the concentration and purity of extracted RNA were assessed to evaluate the suitability of samples for RT-qPCR analysis. The RNA values in the samples varied between 45 and 60 ng/µl. The RNA sample was deemed to be pure when its absorbance was measured in a range of 2.0 to 2.2. The A260/A280 ratio was used to measure the purity of the sample at two distinct wavelengths.

Complementary DNA synthesis

The (EasyScript® One-Step gDNA Removal and cDNA Synthesis Super Mix, TransGen Biotech, China) was used for verifying the cDNA synthesis. Reaction mix, anchored oligo dT, random primer, genomic DNA remover, RNase-free water, reverse transcriptase, and the extra total RNA were added as templates as needed for cDNA synthesis. Three steps are involved in a thermal cycler: the first is 10 minutes at 25 °C, the second is 15 minutes at 42 °C, and the final stage is five seconds at 85 °C to inactivate the enzyme

Primers

The current investigation's primers were developed especially for the Canadian company Alpha DNA Company. When the sequences arrived, they were lyophilized and then diluted with nuclease-free water until they reached a concentration of 10 pmol/µl. Table 1 displays the primers used in the study arranged following their reference sequence.

Table 1: Primers used in the current study.

Primer	Sequence (5′→3′ direction)	Tm (°C)	Product size(bp)
HBB gene			
Forward	GAAGGCTCATGGCAAGAAAG	60	199
Reverse	CACTGGTGGGGTGAATTCTT	60	
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) Gene			
Forward	GAAATCCCATCACCATCTTCCAGG	62	*120*
Reverse	GAGCCCCAGCCTTCTCCATG	60	
miR-361-3p			
Forward	TCCCCCAGGTGTGATTCTGATTT	68	
Reverse	CGAGGAAGAAGACGGAAGAAT	62	
miRNA-U6			
miRU6 F.P.	AGAGAAGATTAGCATGGCCCCT	60	
miRNA-universe R.P.	GCGAGCACAGAATTAATACGAC	62	

Quantitative real-time PCR

The reaction was carried out using a final reaction volume of roughly 20 µl following the manufacturer's instructions using a Real-Time PCR instrument (Rotor-Gene Q, QIAGEN, Germany). It was established that 10 µl of the Master mix containing Syper Green dye would be sufficient to set up the required amount of reactions. To finish the reaction, 1 µl of each forward and reverse primer, 5 µl of nuclease-free water, and 3 µl of cDNA template were added. The temperatures and stages of gene expression are shown in Table 2.

Table 2: Stages and temperature of qRT PCR for U6, GAPDH, *HBB,* and *miR-361-3p* genes

Stage		Temperature °C	Time /Sec.	Cycle
Stage 1	Denaturation	94	60	1
Stage 2	Denaturation	94	5	35
	Annealing	58,56,52	15	
	Extension*	72	20	
Stage 3	Dissociation	65-95	1	1

Statistical analysis

The levels of ferritin, hemoglobin, and white blood cells were compared between groups using the Mann-Whitney U test, a non-parametric test suitable for non-normally distributed data. This analysis was performed using Statistical Package for the Social Sciences (SPSS) version 26. The fold change in gene expression levels of the *HBB* gene and *miR-361-3p* was calculated using the $2^{-\Delta Ct}$ and $2^{-\Delta\Delta Ct}$ methods. Additionally, the correlation between the *HBB* gene and *miR-361-3p* expression levels and the study parameters (ferritin, hemoglobin, and white blood cells) was assessed using Spearman's correlation coefficient in SPSS.

A P-value ≤ 0.05 indicates a statistically significant difference or correlation, while a P-value ≤ 0.01 suggests a highly significant difference or correlation [19, 20].

3. Results

According to age

According to the current study, there were no significant differences between the mean ± SE among age for patients and healthy individuals, which were (11.04 ± 0.47) and 15.74 ± 0.59), respectively, with a P-value of 0.7, as shown in Table 3.

Table 3: Mean ± SE of Age among study groups

Groups	Mean ±SE	P- value
Patients	11.04 ± 0.47	0.7
Control	15.74 ± 0.59	

According to Gender

According to the gender investigations, the percentage of males in the patients and healthy groups was (48%) and (56%), respectively, while the percentage of females was (52%) and (44%), respectively. With a P-value of 0.5, as indicated in Table 4, the findings demonstrated that there were no significant differences among them.

Table 4: Number and percentage of males and females according to this investigation

Gender		Groups		Chi-square	p-value
		patients	control		
Gender	male	24(48%)	28(56%)	0.641	0.5NS
	female	26(52%)	22(44%)		
Total		50	50		

Hematology parameters investigation

Hematological parameters in the current study, such as ferritin, hemoglobin, and white blood cells, do not undergo normal distribution. The median ± SE deviation was measured by the Mann-Whitney U test, as shown in Table 5.

Table 5: Hematology parameters of this study

Parameters	Groups	median± SE	P-value
Ferritin concentration (ng/ml)	Patients	3307.5±548.15	0.001
	Control	45.5±3.66	
Hemoglobin (g/dl)	Patients	8.5±0.17	0.001
	Control	14.2±0.79	
White blood cell (*10⁹/L)	Patients	14.5±1.39	0.001
	Control	6.35±0.22	

*Mann-Whitney U Test used

Molecular indicator result

Tables 6 and 7 illustrated the results of gene expression folding for the *HBB* gene and *miR-361-3p* using two methods Δct and Δct calibrator method that demonstrates folding of the *HBB* gene was (1.909) and *miR-361-3p* was (0.692).

Table 6: Fold Expression of *HBB* gene and their target miRNA according to Δct method.

Group	mean target gene	mean reference gene	Δct	2^{-}Δct	Experimental	fold
			HBB			
Patients	20.02	14.7875	5.233	0.027	1.909	1.909±0.02
Control	21	14.835	6.165	0.014	1	1±0.00
			miR- 361- 3p			
Patients	18.497	12.35	6.147	0.014	0.692	0.692±0.03
Control	18.005	12.39	5.615	0.02	1	1 ± 0.00

Table 7: Fold Expression of *HBB* gene and their target miRNA according to Δct calibrator method.

Group	mean target gene	mean reference gene	Δct	Δct calibrator	ΔΔct	2^{-}ΔΔct	Experimental	fold
HBB								
Patients	20.02	14.7875	5.233	6.905	-1.56	2.954	1.909	1.909
control	21	14.835	6.165	6.905	-0.59	1.505	1	1
miR-361- 3p								
Patients	18.497	12.35	6.147	6.11	0.037	0.975	0.692	0.692
control	18.005	12.39	5.615	6.11	-0.5	1.409	1	1

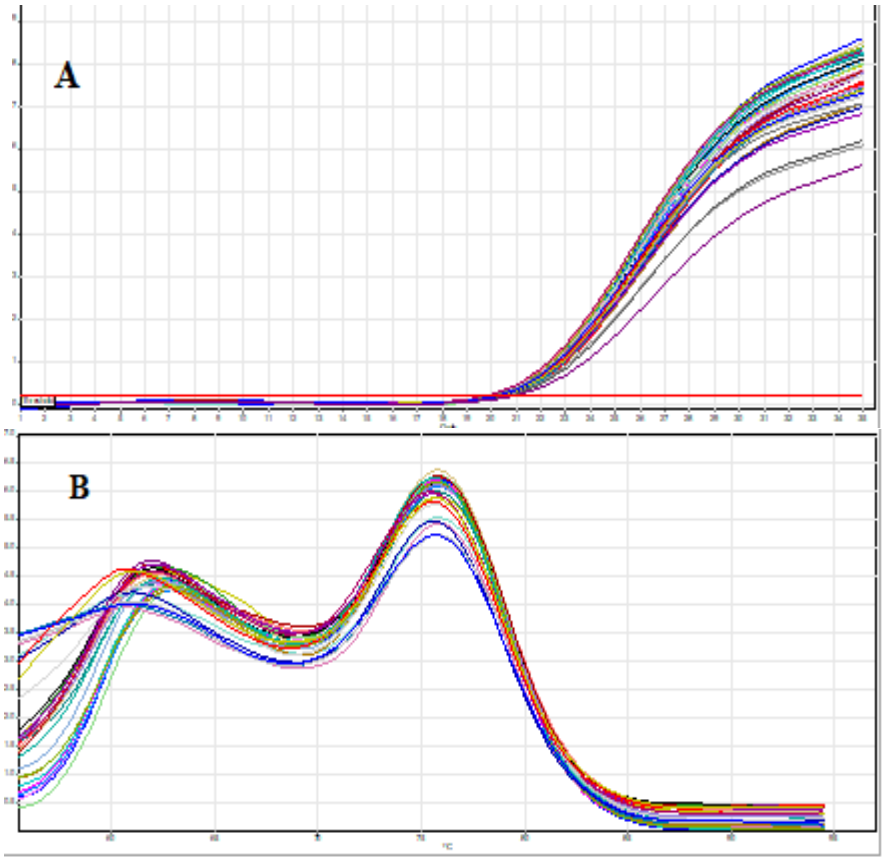

Figure 1: The qRT-PCR output of the *HBB* gene. A: Amplification curve with threshold cycle between (19-21). B: Melting curve.

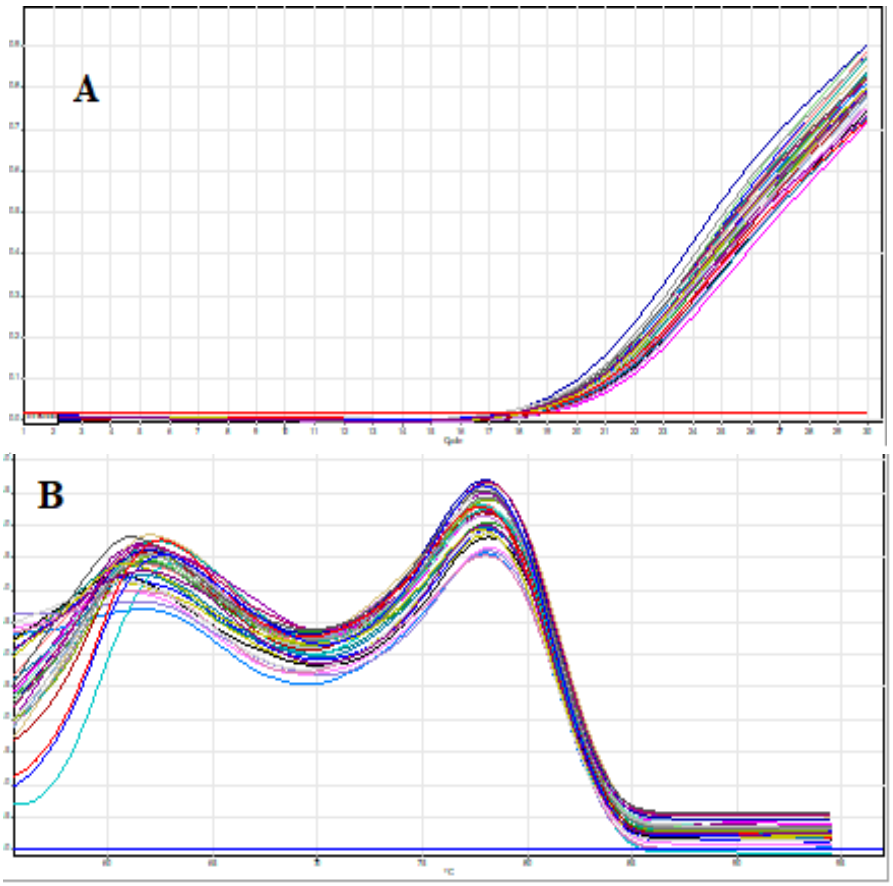

Figure 2: The qRT-PCR output of *miR-361- 3p* gene. A: Amplification curve with threshold cycle between (17-19). Melting curve.

Correlation test

Table 8 and Figure 3 displayed the Spearman correlation coefficient factor that evaluated some parameters of the current study, such as age, ferritin level, hemoglobin, white blood cell (WBC) counts, and gene expression level for both *HBB* and *miR-361-3p*. The study revealed that there is a moderate positive correlation between the *miR-361-3p* gene and both ferritin levels and WBC, where the correlation coefficient values were (0.527) and (0.463), respectively, with highly significant differences, where the P-value was 0.0001. The correlation was negative between *miR-361-3p* and both hemoglobin levels and age, with a highly significant difference and correlation coefficient values of (-0.578) and (-0.311), respectively.

We conclude from this that the level of ferritin and WBC can be modulated by the *miR-361-3p* gene, meaning that the low level of gene expression may be due to the low level of ferritin and WBC. The negative correlation with hemoglobin level may be another indicator that confirms that the *miR-361-3p* gene targets the *HBB* and hence causes deregulation and unpredictability of the gene expression of the *HBB* gene in thalassemia patients.

Table 8: Spearman's rank correlation between parameters in the current study

Parameters	Spearman's rank correlation coefficient	P-value
Age & Ferritin	-0.458**	0.0001**
Age & HGB	0.338**	0.0001**
Age & WBC	-0.188*	0.02*
Age & HBB	0.023	0.7 NS
Age & *miR-361-3P*	-0.311**	0.0001**
Ferritin & HGB	-0.654**	0.0001**
Ferritin & WBC	0.555**	0.0001**
Ferritin & HBB	-0.078	0.3 NS
Ferritin & *miR-361-3P*	0.527**	0.0001**
HGB & WBC	-0.648**	0.0001**
HGB & HBB	-0.072	0.3 NS
HGB & *miR-361-3P*	-0.578**	0.0001**
WBC & HBB	-0.579**	0.0001**
WBC & *miR-361-3P*	0.463**	0.0001**
HBB & *miR-361-3P*	-0.029	0.7 NS

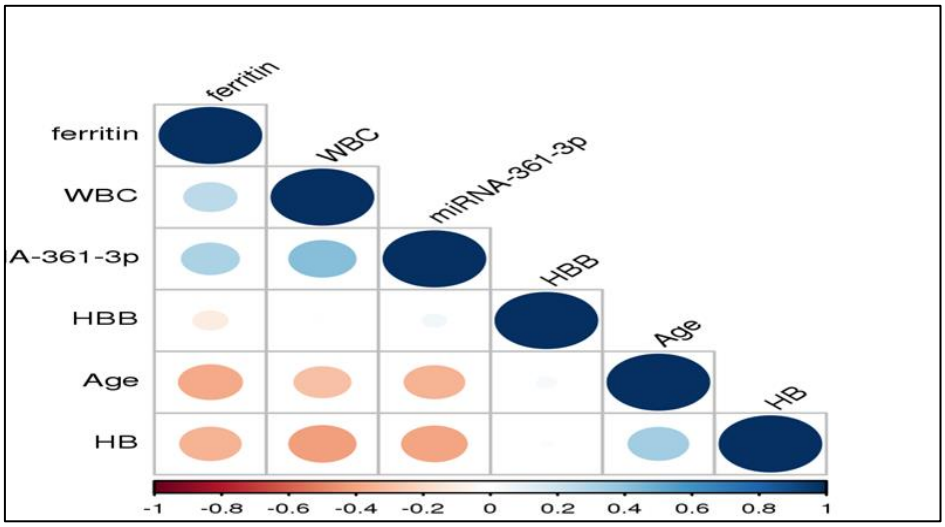

Figure 3: The Spearman correlation coefficient factor among study parameters.

The receiving operating characteristics (ROC) curve was evaluated for both the *HBB* gene and miR-361-3p, where the sensitivity and specificity for the *HBB* gene were (50%) and (74%) respectively, which demonstrated good discriminations, while the sensitivity and specificity for *miR-361-3p* gene were 84% and 92%, respectively that exhibited excellent discrimination, and consequently it may be used as a molecular biomarker to target beta-thalassemia. Table 9 and Figure 4 illustrate the results.

Table 9: Receiving operating characteristics of *HBB* gene and *miR-361-3p* gene

Parameters	AUC	Cut off	Sen%	Sp%	P-value
HBB gene	0.529	20.39	50%	74%	0.5
miR-361-3p	0.918	18.29	84%	92%	0.0001

*AUC: Area under curve, Sen: sensitivity, SP: Specificity.

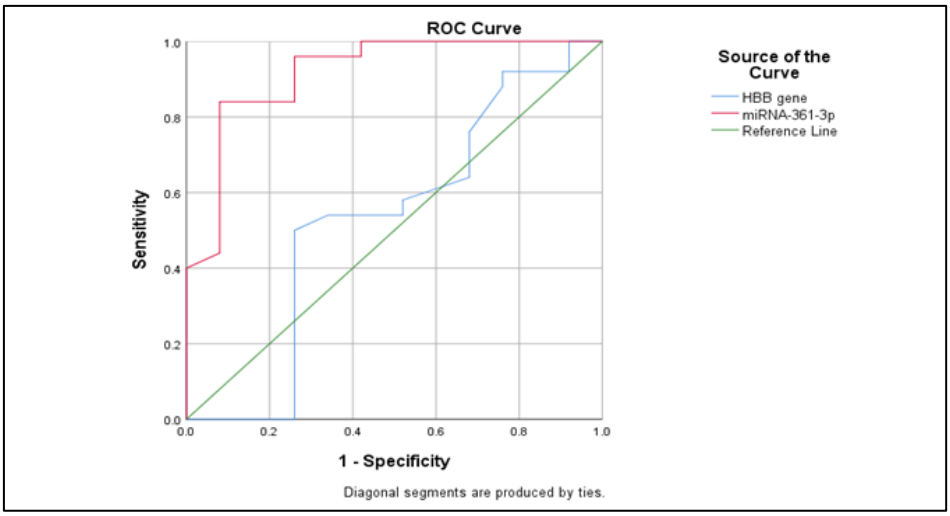

Figure 4: ROC curve of *HBB* gene and *miR-361-3p* gene

4. Discussion

Beta-thalassemia, a hereditary condition distinguished by aberrant hemoglobin synthesis, if untreated, may contribute to health issues, and it is potentially fatal inherited anemia [2]. With novel medicines signaling the start of a new era in thalassemia management, new milestones have been accomplished, but both established and novel strategies need medical knowledge from healthcare professionals, accessibility, affordability, and patient acceptance and adherence [21]. The miRNAs are adaptable modulators of gene expression that are essential for many biological functions. Gaining insight into the nuances and complexity of miRNA regulation is crucial for comprehending the complex networks that control cellular activity and creating innovative treatment strategies for diseases in humans [22]. Ferritin levels were higher in thalassemia patients than in the control group, according to a study conducted by Salih and coworkers in 2023; this finding was consistent with the concept that iron overload acts as a prevalent consequence of thalassemia disorders, enhances the risk of mortality and may cause organ damage. Furthermore, despite the absence of inflammation, there is a positive association between blood ferritin levels and total iron reserves [23].
Thalassemia is a defect that stems from mutations in the genetics that control the synthesis of hemoglobin ("sickle cell anemia"), which leads to a dysfunctional production of red blood cells. There have been reports of the dysregulation of numerous single nucleotide polymorphisms (SNPs) in a patient with thalassemia, which indicates its integration in the discrepancy of disease mechanisms. This means that under the conditions of beta-thalassemia, there is a chance that *miR-361-3p* can be utilized in modulating the expression of *HBB*. Another genetic ailment resulting from a mutation of the *HBB* gene is sickle cell disease (SCD) which also has possible therapeutic uses by concentrating on the specific *miR-361-3p*. In studies performed on SCD mice models, it was demonstrated that the inhibition of *miR-361-3p* resulted in higher expression levels of the *HBB* gene and improved functions of red blood cells. These findings suggest that focusing on *miR-361-3p* could be an effective approach to treating sickle cell disease [24].

The micro RNA produced from endogenous genes has greatly assisted in the regulation of globin expression. In 2021, Wang *et al.,* proposed that miRNA might function as biomarkers in the diagnosis and prediction of the outcomes of patients with β-thalassemia as well as suggesting that a focused therapy that uses miRNA could be employed complementarily with other therapies to target β-thalassemia more efficiently.[6] MiRNA, sometimes called non-

coding RNA, is a type of RNA with a length of 22 bases that does not code for any protein. It represses gene expression by binding to a previously mentioned region situated in the 3'UTR of mRNA or coding RNA. This type of control is thought to occur in 30-50% of all genes. MiRNA uses transcript degradation and translational inhibition as two of its many regulatory strategies [25]. Salih and coworkers investigation, which examined the β-globin promoter gene expression in β-thalassemia patients in the Al-Qadisiyah Governorate, Iraq, verified earlier studies demonstrating that several molecular lesions, such as point mutations, small deletions involving only the *HBB* gene, and large deletions involving the entire globin cluster, can lead to downregulation of the globin gene[23].

The miRNA regulation is a complex process with multiple factors influencing its outcome. Despite computational predictions, experimental validation is essential due to the imprecise nature of miRNA-mRNA interactions. miRNAs form intricate regulatory networks, impacting gene expression dynamically based on various factors. Understanding these complexities is crucial for developing effective therapeutic strategies, especially considering the link between miRNA dysregulation and human diseases [8]. The complex regulatory processes involved in hemoglobin synthesis and the etiology of blood disorders are highlighted by the associations observed between the *HBB* gene and *miR-361-3p*. Targeting these links may have therapeutic benefits, but further investigation must be conducted to completely understand the fundamental molecular pathways[26]. The expression levels of *miR-361-3p* have been previously examined in different health disorders, such as cancer and other blood disorders[16, 17]. The outcomes of the present investigation indicate that beta-thalassemia patients had significantly lower levels of *miR-361-3p* gene expression compared to controls. Subsequent analysis revealed an adverse correlation between *miR-361-3p* and the *HBB* gene expression levels.

Investigators have been studying the possible correlation between the *HBB* gene and miR-361-3p in great detail in the past few years; encoding the beta-globin portion of hemoglobin, a crucial protein accountable for oxygen transport in red blood cells, is the *HBB* gene, which may be found on chromosome 11. The hemoglobinopathies, thalassemia and sickle cell diseases have resulted from the mutations in the *HBB* gene. The miRNA (miRNA) controlled post-transcriptional changes in gene expression. The *miR-361-3p* has been demonstrated to regulate the apoptosis, differentiation, and proliferation of the cells [24, 27,28], which indicated a relation between the *HBB* gene and *miR-361-3p*. For example, *miR-361-3p* directly targets and dysregulates the expression of the *HBB* gene in human red blood cell disorder. Changes in erythroid differentiation and hemoglobin synthesis have been linked to this downregulation [29]. In addition, the function of miR-361-3p in sickle cell disease has also been studied. In that context, a correlation between aberrant hemoglobin synthesis and higher expression of *miR-361-3p* and lower levels of *HBB* gene expression has been confirmed [30]. When a miRNA and the 3'UTR sequence match approximately 70% identity, it is thought that the miRNA can control the expression of a gene, indicating a high degree of regulatory adaptability. As a consequence, a miRNA controls numerous mRNAs, and one mRNA is controlled by multiple miRNAs. The association between miRNA and mRNAs regarding regulation or non-regulation is intricate, involving "many-to-many" interaction [31].

Previous investigations on *miR-361-3p* revealed similar results to the current study in the case of decreased expression level of the gene but in another disorder. For example, Huang and his team's findings in 2021 revealed that *miR-361-3p* was expressed less in tumor tissues and cells of patients with rectal cancer, while Lago and colleagues showed that *miR-361-3p*

was significantly more expressed in lesions from cutaneous leishmaniasis than in samples from normal skin (p =0.0001)[32, 33]. There is previous evidence of elevated expressions of *miR-361-3p* in the t(11;14) translocation in multiple myeloma patients, and the *PPP2R4* gene is the target of this miRNA, which also activates IL-6 signaling and promotes myeloma cell proliferation and survival [34].

The expression of *MIR31HG* and *miR-361-3p* in CRC tissues and normal tissues was analyzed in 2021 by Guo and colleagues using quantitative real-time PCR (qRT-PCR). They discovered that *MIR31HG* was upregulated in CRC tissues. The overexpression of *MIR31HG* inhibited *miR-361-3p*, which suppressed the cancerous activities of CRC cells. Upregulating *MIR31HG* mainly neutralized the anti-tumor effects of *miR-361-3p*. Additionally, the MIR31HG-miR-361-3p-YY1 positive feedback loop accelerated the development of CRC by encouraging the proliferation and glycolysis of CRC cells as well as the angiogenesis of HUVECs [35].

It has been suggested that modulating the expression of miRNA could assist thalassemic patients with lower clinical challenges because it is involved in the production of globin genes and transcriptionally regulates erythroid-specific genes. In general, reactivation of these genes may modify the condition of thalassemic cells and improve the pathophysiology and clinical symptoms of hemoglobinopathies. This would allow for the utilization of these small non-coding RNA as new therapeutic targets, given the significance of miRNA in regulating the expression of globin genes[24]. Changes in erythroid differentiation and hemoglobin synthesis may be linked to this downregulation; also, we investigated whether the function of *miR- 361-3p* in thalassemia may correlate with the aberrant hemoglobin synthesis and increased expression of the *HBB* gene and decreased levels of *miR-361-3p* expression. Further analysis revealed a negative correlation between *miR-361-3p* expression levels and the expression of the *HBB* gene. This suggests that *miR-361-3p* may play a role in the regulation of *HBB* gene expression in the context of beta-thalassemia.
The limited sample size, age range, and thalassemia subtype (beta) represent the major limitations of this investigation. Thus, expanding future studies to include a larger and more comprehensive sample and investigating other types of thalassemia is a critical step toward a deeper understanding of the disease. Furthermore, studying the miRNA expression at the family level may help uncover new genetic factors associated with the disease, thereby opening new avenues for the development of targeted therapies.

5. Conclusions

The current study demonstrated a significant correlation between β-thalassemia and several parameters, including elevated ferritin and white blood cell counts and decreased hemoglobin levels. These findings were attributed to the genetic defect of the disease that results in the abnormal production of red blood cells, necessitating frequent blood transfusions to compensate for the severe deficiency, and the continuous destruction of red blood cells leads to a chronic inflammatory response in the body. Furthermore, genetic analysis revealed decreased expression levels of *miR-361-3p* and increased expression levels of *HBB* in β-thalassemia patients. This suggests a potential role for *miR-361-3p* in regulating the expression of *HBB*. Receiver operating characteristic (ROC) curve analysis indicated that *miR-361-3p* could be a promising biomarker for the diagnosis and monitoring of β-thalassemia patients. However, the current study is limited by its small sample size and focus on a specific disease subtype. Therefore, larger and more comprehensive studies are needed to confirm these findings and evaluate the potential of *miR-361-3p* as a therapeutic target.

Competing interests

The authors declare no competing interests.

Data availability

All data are included in the article.

References

[1] S. Ali, S. Mumtaz, H. A. Shakir, M. Khan, H. M. Tahir, S. Mumtaz, T. A. Mughal, A. Hassan, S. A. R. Kazmi, and Sadia, "Current status of beta-thalassemia and its treatment strategies," *Molecular genetics & genomic medicine,* vol. 9, no. 12, p. e1788, 2021.

[2] I.Z. Sadiq, F.S. Abubakar, H.S. Usman, A.D. Abdullahi, B. Ibrahim, B.S. Kastayal, M. Ibrahim, H.A. Hassan, "Thalassemia: Pathophysiology, Diagnosis, and Advances in Treatment," *Thalassemia Reports*, vol.14, p. 81–102, 2024.

[3] T.-H. Jaing, T.-Y. Chang, S.H. Chen, C.-W. Lin, Y.C. Wen, and C.C. Chiu, "Molecular genetics of β-thalassemia: A narrative review," *Medicine,* vol. 100, no. 45, p. e27522, 2021.

[4] T. Qidwai and T. Qidwai, "Beta-Thalassemia," *Exploration of Host Genetic Factors associated with Malaria,* pp. 43-53, 2021.

[5] M. Filser, B. Gardie, M. Wemeau, P. Aguilar-Martinez, M. Giansily-Blaizot, and F. Girodon, "Importance of sequencing HBA1, HBA2 and HBB genes to confirm the diagnosis of high oxygen affinity hemoglobin," *Genes,* vol. 13, no. 1, p. 132, 2022.

[6] F. Wang, L. Ling, and D. Yu, "MicroRNAs in β-thalassemia," *The American Journal of the Medical Sciences,* vol. 362, no. 1, pp. 5-12, 2021.

[7] T. Abu-Izneid, N. AlHajri, A.M. Ibrahim, M.N. Javed, K.M. Salem, F.H. Pottoo, M.A. Kamal, "Micro-RNAs in the regulation of immune response against SARS CoV-2 and other viral infections, " *Journal of Advanced Research*, vol. 30, p.133–145, 2021.

[8] M. J. Pajares, E. Alemany-Cosme, S. Goñi, E. Bandres, C. Palanca-Ballester, and J. Sandoval, "Epigenetic regulation of microRNAs in cancer: shortening the distance from bench to bedside," *International Journal of Molecular Sciences,* vol. 22, no. 14, p. 7350, 2021.

[9] E. Rykova, N. Ershov, I. Damarov, and T. Merkulova, "SNPs in 3′ UTR miRNA target sequences associated with individual drug susceptibility," *International Journal of Molecular Sciences,* vol. 23, no. 22, p. 13725, 2022.

[10] Z. Han, S. T. Rosen, and C. Querfeld, "Targeting microRNA in hematologic malignancies," *Current opinion in oncology,* vol. 32, no. 5, pp. 535-544, 2020.

[11] A. Lv, M. Chen, S. Zhang, W. Zhao, J. Li, S. Lin, Y. Zheng, N. Lin, L. Xu, and H. Huang, "Upregulation of miR-6747-3p affects red blood cell lineage development and induces fetal hemoglobin expression by targeting BCL11A in β-thalassemia," *Molecular Medicine Reports,* vol. 31, no. 1, p. 7, 2024.

[12] B. Mansoori, B. Baradaran, A. Nazari, F. A. Gaballu, W. C.-S. Cho, and B. Mansoori, "MicroRNAs in the cancer cell-to-cell communication: An insight into biological vehicles," *Biomedicine & Pharmacotherapy,* vol. 153, p. 113449, 2022.

[13] B. Hua, Y. Li, X. Yang, X. Niu, Y. Zhao, and X. Zhu, "MicroRNA-361-3p promotes human breast cancer cell viability by inhibiting the E2F1/P73 signalling pathway," *Biomedicine & Pharmacotherapy,* vol. 125, p. 109994, 2020.

[14] X. Li and H. Zheng, "LncRNA SNHG1 influences cell proliferation, migration, invasion, and apoptosis of non-small cell lung cancer cells via the miR-361-3p/FRAT1 axis," *Thoracic Cancer,* vol. 11, no. 2, pp. 295-304, 2020.

[15] M. Karssli, "CRISPR technology in human medicine: advantages and disadvantages over traditional treatments," Hochschule Rhein-Waal, 2022.

[16] Z. Fan, Z. Wu, and B. Yang, "The effect of miR-361-3p targeting TRAF6 on apoptosis of multiple myeloma cells," *Journal of Microbiology and Biotechnology,* vol. 31, no. 2, p. 197, 2020.

[17] H. Zhou, H. Tang, N. Li, H. Chen, X. Chen, L. Gu, L. Zhang, G. Tian, and D. Tao, "MicroRNA-361-3p inhibit the progression of lymphoma by the Wnt/β-catenin signaling pathway," *Cancer Management and Research,* pp. 12375-12384, 2020.

[18] F. Longo, A. Piolatto, G. B. Ferrero, and A. Piga, "Ineffective erythropoiesis in β-thalassaemia: key steps and therapeutic options by drugs," *International Journal of Molecular Sciences,* vol. 22, no. 13, p. 7229, 2021.

[19] A. Alili and D. Krstev, "Using spss for research and data analysis," *Knowledge–International Journal,* vol. 32, no. 3, 2019.

[20] M. Faeqali Jan, H. Muneer Al-Khafaji, B. Hasan Al-Saadi, M.K. Aneed Al-Saedi, "Assessment of Interleukin-8 in Bronchial Asthma in Iraq," *Archives of Razi Institute, vol.* 76, p. 913–923, 2021

[21] M. Angastiniotis, "Beta thalassemia: Looking to the future, addressing unmet needs and challenges," *Annals of the New York Academy of Sciences,* vol. 1532, no. 1, pp. 63-72, 2024.

[22] A. A. Seyhan, "Trials and tribulations of MicroRNA therapeutics," *International Journal of Molecular Sciences,* vol. 25, no. 3, p. 1469, 2024.

[23] M. M. Salih, A. G. Al-Ziaydi, and A. H. H. Alzamili, "The Effect of Ferritin Level and Gene Expression of β-globin Promoter with β-thalassemia Patients in Al-Qadisiyah Governorate, Iraq," *Journal of Applied Hematology,* vol. 14, no. 4, pp. 257-262, 2023.

[24] N. Saki, S. Abroun, M. Soleimani, M. Kavianpour, M. Shahjahani, J. Mohammadi-Asl, and S. Hajizamani, "MicroRNA expression in β-thalassemia and sickle cell disease: a role in the induction of fetal hemoglobin," *Cell Journal (Yakhteh),* vol. 17, no. 4, p. 583, 2016.

[25] P. Yadav, R. Tamilselvan, H. Mani, K.K. Singh, "MicroRNA-mediated regulation of nonsense-mediated mRNA decay factors: Insights into microRNA prediction tools and profiling techniques," *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, vol. 1867 , p.195022, 2024.

[26] M.D. Diamantidis, G. Ikonomou, I. Argyrakouli, D. Pantelidou, S. Delicou, "Genetic Modifiers of Hemoglobin Expression from a Clinical Perspective in Hemoglobinopathy Patients with Beta Thalassemia and Sickle Cell Disease," *International Journal of Molecular Sciences*, vol. 25, no.22, p. 11886 ,2024.

[27] M. Ratti, A. Lampis, M. Ghidini, M. Salati, M. B. Mirchev, N. Valeri, and J. C. Hahne, "MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) as new tools for cancer therapy: first steps from bench to bedside," *Targeted oncology,* vol. 15, pp. 261-278, 2020.

[28] C. E. Condrat, D. C. Thompson, M. G. Barbu, O. L. Bugnar, A. Boboc, D. Cretoiu, N. Suciu, S. M. Cretoiu, and S. C. Voinea, "miRNAs as biomarkers in disease: latest findings regarding their role in diagnosis and prognosis," *Cells,* vol. 9, no. 2, p. 276, 2020.

[29] Y. Chen, T. Li, Q. Gao, L.-Y. Wang, and L.-Q. Cui, "MiR-1908 improves cardiac fibrosis after myocardial infarction by targeting TGF-β1," *European Review for Medical & Pharmacological Sciences,* vol. 22, no. 7, 2018.

[30] W. Chen, J. Wang, S. Liu, S. Wang, Y. Cheng, W. Zhou, C. Duan, and C. Zhang, "MicroRNA-361-3p suppresses tumor cell proliferation and metastasis by directly targeting SH2B1 in NSCLC," *Journal of Experimental & Clinical Cancer Research,* vol. 35, pp. 1-16, 2016.

[31] L. F. Gulyaeva and N. E. Kushlinskiy, "Regulatory mechanisms of microRNA expression," *Journal of translational medicine,* vol. 14, no. 1, p. 143, 2016.

[32] F. Huang, H. Li, Z. Qin, A. Wang, Y. Zhang, J. Guo, M. Wei, H. Guo, and J. Pu, "SNHG17 serves as an oncogenic lncRNA by regulating the miR-361-3p/STC2 axis in rectal cancer," *Frontiers in genetics,* vol. 12, p. 654686, 2021.

[33] T. S. Lago, J. A. Silva, E. L. Lago, E. M. Carvalho, D. L. Zanette, and L. C. Castellucci, "The miRNA 361-3p, a regulator of GZMB and TNF is associated with therapeutic failure and longer time healing of cutaneous leishmaniasis caused by L.(viannia) braziliensis," *Frontiers in Immunology,* vol. 9, p. 2621, 2018.

[34] M. Lionetti, M. Biasiolo, L. Agnelli, K. Todoerti, L. Mosca, S. Fabris, G. Sales, G. L. Deliliers, S. Bicciato, and L. Lombardi, "Identification of microRNA expression patterns and definition of a microRNA/mRNA regulatory network in distinct molecular groups of multiple myeloma," *Blood, The Journal of the American Society of Hematology,* vol. 114, no. 25, pp. e20-e26, 2009.

[35] T. Guo, D. Liu, S. Peng, M. Wang, and Y. Li, "A positive feedback loop of lncRNA MIR31HG-miR-361-3p-YY1 accelerates colorectal cancer progression through modulating proliferation, angiogenesis, and glycolysis," *Frontiers in oncology,* vol. 11, p. 684984, 2021.