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The Effect of Ferritin Level and Gene Expression of β-globin Promoter with β-thalassemia Patients in Al-Qadisiyah Governorate, Iraq

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Abstract:

BACKGROUND: The genetic condition β-thalassemia causes a deficit in the β-globin chain. Goblins are produced under the supervision of at least nine different genes. Thalassemia can be distinguished from other disorders by changes in these genes, which can lead to issues with hemoglobin synthesis. A typical side effect of thalassemia syndromes is iron overload, which raises the risk of mortality and can cause organ damage. Blood ferritin levels as well as total iron of body reserves have a positive correlation when there is no inflammation.

OBJECTIVE: The purpose of this study was to assess the ferritin level of an Iraqi patient and the relationship between β-thalassemia and gene expression of β-globin.

MATERIALS AND METHODS: A case–control study included 60 samples with mean age (17.76 ± 0.88; 28 males and 32 females) which had been collected from patients who were diagnosed with β-thalassemia and 60 samples with mean age (22.7 ± 0.75; 29 males, 31 females) which were collected from apparently healthy individuals as a control group (CG). The procedure's outcome is monitored using polymerase chain reaction and the Fluorecare instrument.

RESULTS: Ferritin levels in thalassemia patients were higher than in CG patients. The β-globin expression in the thalassemia group was significantly lower than in the CG. The discovery of two essential sequences thymine-adenine-thymine-adenine and cytosine-adenine-thymine-adenine in the β-gene promoter that are crucial in the start of transcription can account for this downregulation. Changes made to these sequences decreased the affinity of transcription factors, which in turn restricted the transcription of the messenger ribonucleic acid. Examples of these transcription factors are erythroid Kruppel-like factor and specificity protein 1.

CONCLUSION: Ferritin can be a useful indicator of severe iron overload. The results showed that the level of expression of β-globin was dramatically downregulated within the thalassemia group as compared with the CG future prospective of this study.

Keywords:

Globin chain, real-time polymerase chain reaction, ribonucleic acid extraction, thalassemia illness

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Introduction

Four β-globin chains make the primary adult hemoglobin A (HbA), a tetramer comprising two molecules. A partial or total lack in the synthesis of these chains results in thalassemia, an autosomal recessive form of chronic hemolytic anemia. A single or multiple of the many mutations in the important genes is what causes it.[1] Unpaired globin chains, which are prone to instability and intracellular precipitation, are the reason why red blood cell (RBC) precursors in the bone marrow undergo apoptosis and adult RBCs have a short lifespan in the circulatory system.[2] Iron, hemi.[3] Changes in these genes, which may

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cause hemoglobin synthesis difficulties and distinguish thalassemia from other disorders.[4] The indications of β-thalassemia include low levels of HbA when hemoglobin is analyzed, microcytic hypochromic anemia, and an abnormal peripheral blood smear with nucleated RBC.[5] Thalassemia intermedia patients present later in life and have a less severe anemia that does not require frequent blood transfusions. These people are at risk for iron overload due to insufficient erythropoiesis, which is brought by increased intestinal iron absorption.[6] The development of organ damage and greater mortality rates have been linked to iron overload, a common side effect of thalassemia syndromes.[7] These people begin to experience iron accumulation in parenchymal tissues around a year of beginning the routine transfusions.[8-10] In addition, people who have nontransfusion-dependent thalassemia may develop iron overload.[11] Compared to the healthy people, the enhanced gastrointestinal absorption of iron is substantially higher which is probably caused by the paradoxical decreased hepcidin in thalassemia intermedia.[12] Two genes from each parent are responsible for producing β-globin protein chains. If the individual has one gene defect, one has mild signs and symptoms, and this condition is called β-thalassemia minor or a β-thalassemia trait. The patients with two genes and five abnormalities will have mild to severe symptoms, this illness is known as thalassemia major (TM) or Cooley's anemia.[13,14] 150 million people or 3% of the world's population are thought to be carriers of the β-thalassemia gene. These carriers or those with the characteristics of β-thalassemia are mostly normal. Hence, following screening red cell indices that reveal a decrease in mean corpuscular volume and hemoglobin value. They are typically identified by testing an elevated hemoglobin A2 level. The range of molecular abnormalities in the β-globin gene that cause β-thalassemia has been better understood over the last 8 years, suggesting that 99% of the world's known β-thalassemia genes are caused by gene abnormalities.[15]

Hemoglobin switching is the process by which an embryo develops into a fetus and subsequently into an adult by manufacturing-like globin genes at various stages of development. Adult hemoglobin becomes the predominant hemoglobin and mutations affecting the adult hemoglobin subunit beta (HBB) gene of β-thalassemia.[16] The human β-globin gene locus (11p) on chromosome 11 contains the globin genes, which are among the most thoroughly investigated and well-characterized gene loci in the human genome. This locus's genes are arranged like a telomere centromere Gg-Ag-d-b.[17] Therefore, the purpose of this study was to assess the relationship between β-thalassemia and gene expression using biochemical and molecular biology instruments reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to achieve the target results.

Materials and Methods

A case–control study of 120 patients at the Al-Diwaniyah Teaching Hospital and the local β-thalassemia clinic provided samples. Sample collection began from November 2021 to May 2022, including (60) healthy volunteers for the control group (CG) (G1) and (60) thalassemia patients (G2). Five milliliters of venous blood was drawn, 2 mL was immediately placed in dipotassium ethylenediaminetetraacetic acid Vacutainer tubes, and 3 mL was drawn into gel tubes, which help with the appropriate separation of the serum. The serum and whole blood were kept frozen at −80°C. The blood sample was centrifuged (4000 × *g*) at room temperature and 10 min. To evaluate various features, samples were separated into Eppendorf containers as well as refrigerated at −80°C.

Measurement of ferritin concentration

We employed a Fluorecare equipment, model MF-T1000, and immunofluorescence kit from Microprofit BioTech, China, for this test.

Measurement of the gene expression of β-globin promoter

A positive reaction in a real-time polymerase chain reaction experiment is identified by the buildup of a fluorescent signal. The cycle threshold (Ct) is the number of cycles required for the fluorescent signal to reach the threshold (i.e., to exceed the background level). Ct levels are inversely related to the amount of target nucleic acid in the sample (i. e., the lower the Ct level, the more target nucleic acid in the sample). Cts <29 are strong positive reactions indicative of abundant target nucleic acid in the sample; Cts of 30–37 are positive reactions indicative of moderate amounts of target nucleic acid; Cts of 38–40 are weak reactions indicative of minimal amounts of target nucleic acid which could represent an infection state or environmental contamination. The Ct method delta delta Ct (Ct) was used to calculate the quantities of actin messenger ribonucleic acid (mRNA) transcripts and normalize them to the CG level. The globin gene was amplified using the shown primers, and the actin gene was amplified to serve as a housekeeping gene [Table 1].[18]

Quantitative reverse transcription real-time PCR (RT-qPCR)

Two hundred microliters of total RNA was reverse transcribed for gene expression investigation using the TaqMan® Reverse Transcription Reagents kit and random hexamers. The RT-qPCR experiment employed gene-specific double fluorescently tagged probes. Prime Time® Gene Expression Master Mix 1X was used in the reaction mixture, which had a final volume of 20 μL. Forward and reverse primer pairs (β-globin-F,

Table 1: Primers for polymerase chain reaction

Primer name	Sequence '5–3'	Target gene
β-globin-F	ATCCTGAGAACTTCAGGCTCCTGGG	Gene of interest
β-globin-R	GAGCTTAGTGATACTTGTGGGCCAG	
β-actin-F	CCACACTGTGCCCATCTACG	Internal reference gene (normalizer)
β-actin-R	CCGTGGTGGTGAAGCTGTAG	

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β-globin-R, β-actin-F, and β-actin-R) were used at 1 μL for each concentration. After a 2-min denaturation step at 95°C, the reactions were run for 40 cycles of 15 s at 95°C, 60 s at 60°C, 35 s at 60°C, and 15 s at 60°C[19] (this experiment was carried out at the ADD BIO Inc., South Korea).

Ethical considerations

A written illustrative consent form was signed by all parents/caregivers of the participating patients. This study was performed according to the ethical rules for medical research involving human participants of the Declaration of Helsinki (1964). Ethical approval was received from the Ethical and Research Committee of the Department of Medical Chemistry, College of Medicine, University of Al-Qadisiyah, Iraq.

Statistical analysis

The statistical analysis of the data was done by the Statistical Package for the Social Sciences (SPSS) version 22 (2013), (IBM, SPSS Inc, USA). For parametric variables, an independent sample *t*-test was used, and a Mann–Whitney U-test was used for nonparametric variables. The correlation between dependent variables was evaluated using Pearson's correlation coefficient analysis. The result was statistically significant when $P \leq 0.05$.

Results

Serum level of ferritin in β-thalassemia patients

The present study clarified a significant increase in ferritin in comparison with the CG in patients having β-thalassemia ($P < 0.0001$), as shown in Figure 1 and Table 2.

Curves for the promoter and amplification of β-globin

Figure 2a and b demonstrates the effectiveness of β-globin promoter amplification. This demonstrates that the RNA extraction and cDNA synthesis procedures were successful. The internal reference gene β-actin is used as a normalizer in the analysis of the amplification curves for the tested samples.

Melting curve analysis

Figure 3a and b shows the melting curve analysis of the amplified products of the β-globin promoter gene (Interested gene). It exhibits a high level of specific amplification in the absence of primer dimers or nonspecific reactions.

Analysis of the RT-qPCR gene expression data β-globin promoter gene

Comparison of fold changes between controls and patients expressing the globin promoter gene. This shows a significant downregulation in the expression of β-globin in the thalassemia group compared to the CG. The average fold change in expression of the β-globin promoter gene in the comparing groups is 2.3 in the CG and 0.12 in thalassemia patients [Figure 4].

Discussion

In the present study, the patients with β-thalassemia exhibited a significantly elevated concentration of ferritin in comparison to healthy subjects. A typical side effect of thalassemia syndromes is iron overload, which raises the risk of organ damage and increased death. The findings of the current study were consistent with those of the earlier investigation,[20] When thalassemia patients' ferritin levels were compared to normal people, they found higher ferritin levels. Elevation of serum ferritin (SF) in the patients with β-thalassemia and other hemoglobinopathies can be due to long-term gastrointestinal absorption instead of iron supplementation in the short term.[21] With the passage of time, repeated blood transfusion can lead to liver disorders due to the accumulation of iron in hepatocytes. Along with the transaminases (alanine transaminase, aspartate aminotransferase, and gamma-glutamyl transferase), SF can be regarded as an additional type of liver function test in liver disease because injured hepatocytes leak ferritin into the blood circulation, leading to elevated levels of ferritin.[22] While anemia patients benefit greatly from blood transfusions, prolonged transfusions invariably result in iron overload since humans are unable to effectively eliminate excess iron.[23] A structural gene that codes for β-globin is located on chromosome 11 in a cluster with other β-like genes.[24] Five functional genes make up the cluster: ε hemoglobin E, Gγ hemoglobin subunit gamma 2, Aγ hemoglobin subunit gamma 1, δ hemoglobin subunit delta, and β (HBB). These genes are positioned on the chromosome in accordance with the order in which their expression occurs during development to produce distinct Hb tetramers.[25] The nondeletion mutations that can be found within the gene or in the sequences

Table 2: The serum levels of ferritin in β-thalassemia patients

Parameter	Statistics	Control group	Patient group	*P*
Ferritin (ng/mL)	Mean	101.1	4351.10	<0.0001
	Median	89.5	3322.5	
	SD	41.17	3345.26	
	SE	5.823	473.092	
	Minimum	55	328	
	Maximum	234	12,000	
	Range	179	11672	
	IQR	21	5449.7	
	The coefficient of variation	40.60	76.88	
	Kolmogorov–Smirnov[a]	0.000	0.000	
	Shapiro–Wilk	0.000	0.000	

[a]Lilliefors significance correction. SD=Standard deviation; SE=Standard error; IQR=Interquartile range

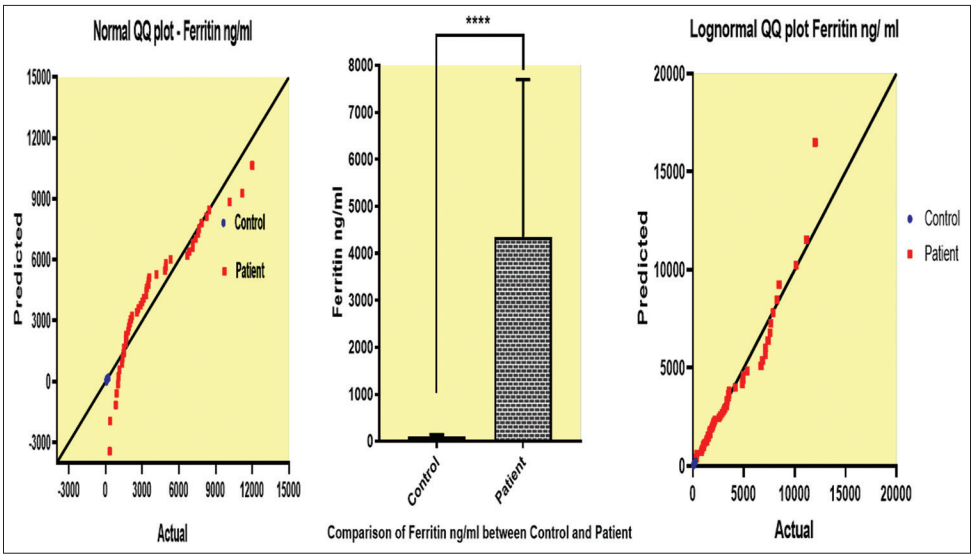

Figure 1: Comparison of the ferritin levels ng/mL between control and patient groups

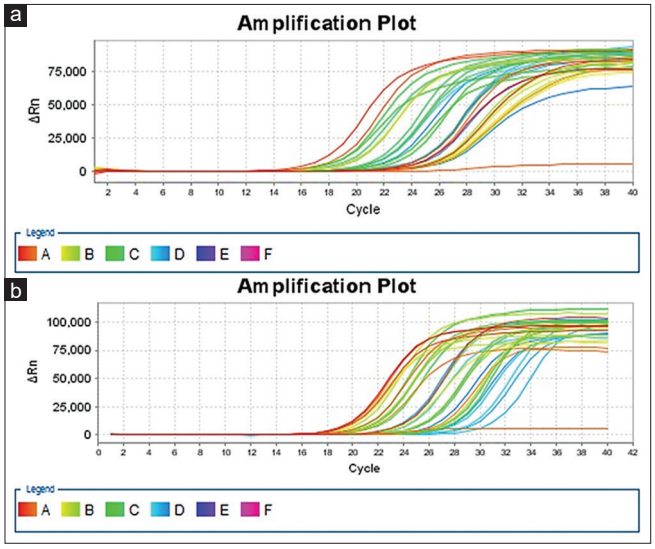

Figure 2: (a) The amplifying curve of the investigated samples represents the β-globin promoter gene. (b) Amplification curves of the examined samples with the internal reference gene β-actin serving as a normalizer for the experiment

that immediately surround it include minor insertions and deletions of one to several bases, as well as single base replacements. They suppress the β-globin gene at nearly every known step of its expression, such as mRNA translation, RNA processing, and transcription. β^0 thalassemia is caused by approximately 50% of nondeletion mutations that totally inactivate the β-gene and prevent the production of β-globin.[26] A feature of β^+-thalassemia is reduced β chain synthesis, which is brought by changes in the promoter region (CACCC or thymine-adenine-thymine-adenine [TATA] box). A diagnosis of mild or silent sickness is made according to the degree of β-globin chain reduction.[27,28] The results of our investigation showed that β-globin expression of the thalassemia group was dramatically downregulated in comparison with the CG. Two sequences are essential for the initiation of transcription in the β-gene promoter: the TATA box, which establishes the starting location, and the duplicates of CACCCC (distal and proximal), which establish the starting frequency. Changes to these regions inhibit the transcription of mRNA by lowering the binding affinity of transcription factors including specificity protein 1 and erythroid Kruppel-like factor.[29] The current study's findings supported

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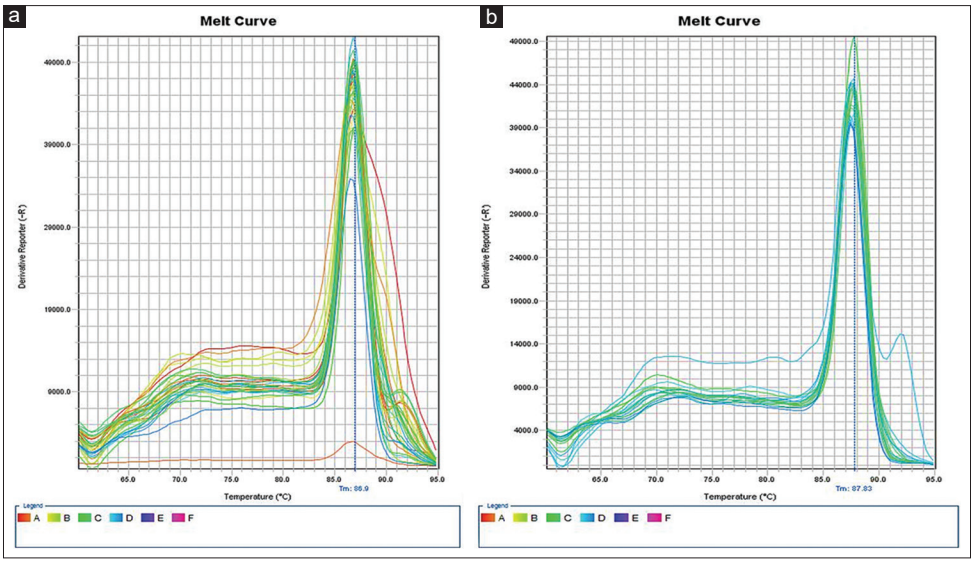

Figure 3: (a) Analysis of the melting curves of the amplified products of the gene of interest, the β-globin promoter. (b) Melting curve study of β-actin amplification products as internal reference gene

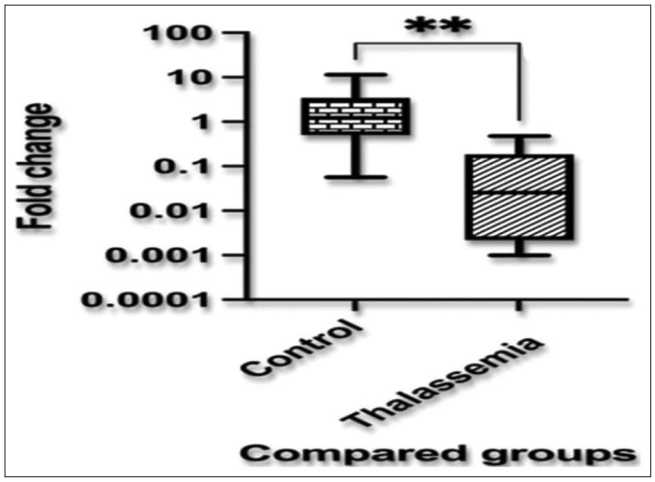

Figure 4: Comparison of fold changes between controls and patients expressing the globin promoter gene

previous research which showed that the globin gene can be downregulated by a variety of molecular lesions, such as point mutations, small deletions of only the HBB gene, and large deletions of the entire globin cluster. A comparative CT method (ΔΔCt) used reference gene and gene of interest to determine the relative quantity of target nucleic acid sequence in samples where ΔΔCt = ΔCt (sample) – ΔCt (reference gene).[30] In the current study, our technique revealed that patients with thalassemia had increased expression of the interest gene when compared to the internal reference gene.

Conclusion

The findings revealed that β-globin expression was significantly lower in the thalassemia group compared to the CG in Iraqi patients. Request for a meritorious investigation of the occurrence and genetic role of TM patients in AlQadisiyah governorate, Iraq.

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Conflicts of interest

There are no conflicts of interest.

References

1. Agarwal S, Gulati R, Singh K. Hemoglobin E-beta thalassemia in Uttar Pradesh. Indian Pediatr 1997;34:287-92.
2. Cao A, Galanello R. Beta-thalassemia. Genet Med 2010;12:61-76.
3. Fibach E, Rachmilewitz EA. Pathophysiology and treatment of patients with beta-thalassemia – An update. F1000Res 2017;6:2156.
4. Al-Mehana W, Mahdi I, Hayder T, Abusaiba H. Gene therapy by studying the effect of single nucleotide polymorphism (rs11886868 and rs766432) at the BcL11A gene on the severity of beta-thalassemia disease in the province of AL-Najaf. Int J Pharm Res 2021;13:3869-75.
5. Ali S, Mumtaz S, Shakir HA, Khan M, Tahir HM, Mumtaz S, *et al*. Current status of beta-thalassemia and its treatment strategies. Mol Genet Genomic Med 2021;9:e1788.
6. Motta I, Bou-Fakhredin R, Taher AT, Cappellini MD. Beta thalassemia: New therapeutic options beyond transfusion and iron chelation. Drugs 2020;80:1053-63.
7. Domellöf M, Dewey KG, Lönnerdal B, Cohen RJ, Hernell O. The diagnostic criteria for iron deficiency in infants should be

reevaluated. J Nutr 2002;132:3680-6.
8. Gibson RS. Principles of Nutritional Assessment. USA: Oxford University Press; 2005.
9. Taher AT, Cappellini MD, Musallam KM. Recent advances and treatment challenges in patients with non-transfusion-dependent thalassemia. Blood Rev 2012;26 Suppl 1:S1-2.
10. Al-Hameedawi AK, Al-Shawi AA. Identification of novel mutations in β-thalassemia patients in Maysan Governorate, Iraq. Mol Biol Rep 2023;50:3053-62.
11. Gardenghi S, Grady RW, Rivella S. Anemia, ineffective erythropoiesis, and hepcidin: Interacting factors in abnormal iron metabolism leading to iron overload in β-thalassemia. Hematol Oncol Clin North Am 2010;24:1089-107.
12. Mamedov AA, Dybov AM, Morozova NS, Kharke VV, Byzov NA. Assessing the levels of demands and needs for comprehensive rehabilitation of patients with congenital and acquired maxillofacial deformities. Syst Rev Pharm 2020;11:50-7.
13. Neishabury M, Zamani F, Keyhani E, Azarkeivan A, Abedini SS, Eslami MS, *et al.* The influence of the BCL11A polymorphism on the phenotype of patients with beta thalassemia could be affected by the beta globin locus control region and/or the Xmn1-HBG2 genotypic background. Blood Cells Mol Dis 2013;51:80-4.
14. Colaco S, Nadkarni A. Borderline HbA (2) levels: Dilemma in diagnosis of beta-thalassemia carriers. Mutat Res Rev Mutat Res 2021;788:108387.
15. Thein SL. Molecular basis of β thalassemia and potential therapeutic targets. Blood Cells Mol Dis 2018;70:54-65.
16. Mettananda S, Higgs DR. Molecular basis and genetic modifiers of thalassemia. Hematol Oncol Clin North Am 2018;32:177-91.
17. Carrocini GC, Zamaro PJ, Bonini-Domingos CR. What influences Hb fetal production in adulthood? Rev Bras Hematol Hemoter 2011;33:231-6.
18. AL-Zubaidi MM, Alkhtaua KJ. Assessment of serum ferritin levels in thalassemia and non-thalassemia patients presented with anemia. Iraqi J Hematol 2015;4:119-25.
19. Zuccato C, Cosenza LC, Zurlo M, Lampronti I, Borgatti M, Scapoli C, *et al.* Treatment of erythroid precursor cells from β-thalassemia patients with cinchona alkaloids: Induction of fetal hemoglobin production. Int J Mol Sci 2021;22:13433.
20. Rujeerapaiboon N, Tantiworawit A, Piriyakhuntorn P, Rattanathammethee T, Hantrakool S, Chai-Adisaksopha C, *et al.* Correlation between serum ferritin and viral hepatitis in thalassemia patients. Hemoglobin 2021;45:175-9.
21. Pokhrel NB, Khanal S, Chapagain P, Pokhrel B, Shrestha A. Hemochromatosis in a β-thalassemia minor patient with H63D homozygous mutation: A case report. Clin Case Rep 2020;8:2341-5.
22. Piñero F, Dirchwolf M, Pessôa MG. Biomarkers in Hepatocellular Carcinoma: Diagnosis, Prognosis and Treatment Response Assessment Cells. 2020;9:1370.
23. Abraham A. Bull's eye maculopathy possibly due to iron overload in a child with thalassemia major: A case of possible "ferritin retinopathy". Retin Cases Brief Rep 2021;15:482-5.
24. Orkin SH. Molecular medicine: Found in translation. Med 2021;2:122-36.
25. Lee JS, Cho SI, Park SS, Seong MW. Molecular basis and diagnosis of thalassemia. Blood Res 2021;56:S39-43.
26. Jaing TH, Chang TY, Chen SH, Lin CW, Wen YC, Chiu CC. Molecular genetics of β-thalassemia: A narrative review. Medicine (Baltimore) 2021;100:e27522.
27. Martelli F, Verachi P, Zingariello M, Mazzarini M, Vannucchi AM, Lonetti A, *et al.* hGATA1 under the control of a μLCR/β-globin promoter rescues the erythroid but not the megakaryocytic phenotype induced by the gata1 (low) mutation in mice. Front Genet 2021;12:720552.
28. Tripathi P, Agarwal S, Tewari S, Mandal K. Status of catalase, glutathione peroxidase, glutathione S-transferase, and myeloperoxidase gene polymorphisms in beta-thalassemia major patients to assess oxidative injury and its association with enzyme activities. J Pediatr Genet 2022;11:198-212.
29. Ropero P, Erquiaga S, Arrizabalaga B, Pérez G, de la Iglesia S, Torrejón MJ, *et al.* Phenotype of mutations in the promoter region of the β-globin gene. J Clin Pathol 2017;70:874-8.
30. Morzaev D, Nicholson JD, Caspi T, Weiss S, Hochhauser E, Goldenberg-Cohen N. Toll-like receptor-4 knockout mice are more resistant to optic nerve crush damage than wild-type mice. Clin Exp Ophthalmol 2015;43:655-65.

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