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The Estimation of IL-7 and IL-37 in Thalassemia Through RNA Sequencing of Peripheral Blood Mononuclear Cells

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Abstract :

Thalassemia is a hematological disorder caused by mutations in the hemoglobin gene that affected the red blood cells .The immune systems of the patients are continuously strained by these repeated conditions. Few studies have examined the immunological status of people with thalassemia, the peripheral blood mononuclear cells (PBMCs) as well as the levels of IL-7 and IL-37 was examined to study the immunological profiles of patients with thalassemia. Also the total RNA were extracted and carried out the RNA sequencing after PBMCs were isolated, to find the differences in gene expression between the patients and the healthy controls. This study showed that the patients with thalassemia had significant higher levels of IL-7 and IL-37 with morphological abnormalities of PBMCs.

Keywords: IL-7 ,IL-37, PBMCs ,RNA sequences,Thalassemia.

تقدير مستويات IL-7 وIL-37 في الثلاسيميا من خلال تسلسل الحمض النووي الريبي للخلايا أحادية النواة في الدم المحيطي

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مستخلص:

الثلاسيميا هو اضطراب دموي ناتج عن طفرات في جين الهيموجلوبين تؤثر على خلايا الدم الحمراء. تتعرض أجهزة المناعة لدى المرضى لضغوط مستمرة بسبب هذه الحالات المتكررة. وقد قامت دراسات قليلة بفحص الحالة المناعية للأشخاص المصابين بالثلاسيميا، وتم فحص خلايا الدم المحيطية وحيدة النواة (PBMCs) وكذلك مستويات IL-7 و IL-37 لدراسة الملامح المناعية لمرضى الثلاسيميا. كما تم استخراج الحمض النووي الريبي الكلي وإجراء تسلسل الحمض النووي الريبي بعد عزل خلايا الدم المحيطية وحيدة النواة، لإيجاد الاختلافات في التعبير الجيني بين المرضى وضوابط الأصحاء. أظهرت هذه الدراسة أن مرضى الثلاسيميا لديهم مستويات أعلى بشكل ملحوظ من IL-7 و IL-37 مع تشوهات مورفولوجية لخلايا الدم المحيطية وحيدة النواة.

الكلمات المفتاحية: الثلاسيميا، خلايا الدم المحيطية وحيدة النواة، تسلسل الحمض النووي الريبي، IL-7، IL-37 .

1. Introduction

A genetic disease called thalassemia is characterized by aberrant hemoglobin synthesis, which lowers the hemoglobin and red blood cell (RBC) levels. The anemia, jaundice, bone abnormalities, organ enlargement, cardiomyopathy, and other problems are only a few of the many clinical indications of thalassemia [1]. According to the World Health Organization (WHO), the illness affects almost 270 million individuals. The Iraqi Ministry of Health (2019) estimates that 22,000 persons suffer from thalassemia [2]. The disease's significance stems from its difficulty is being cured; the only viable treatment for patients is steam cell transplantation, which is scarce because of a reduction in the donors [3]. Immunological distress can also result from iron excess, a prevalent issue in thalassemia. Splenectomy, which is occasionally some thalassemia patients suffering from it, could leading to abnormalities in the immune system [4]. The lymphocyte cells comprise the dendritic cells , macrophage, and monocyte, are the first line of defense against the thalassemia disease [5]. In order to help with the diagnosis and treatment of this disease, the PBMCs are now essential for comprehending the molecular mechanisms underlying a variety of diseases [6]. However, a fewer earlier studies have documented the immune cell alterations. The control of immunity and hemopoiesis depends on the cytokines which are physiologically active compounds that regulate the immune system, inflammation, and hemopoiesis [4] . Mostly, at least 30 cytokines control hemopoiesis; some, like erythropoietin, are produced in response to specific stimuli, while others, like IL37 and IL-7 are present continuously [7]. The IL-7 could keep the immune system in balance by activating the IL-7 receptor (IL-7R),as wheel as the IL-7 had a great role in the growth of T-cell, its development , and its proliferation as well as the B cell development .The IL-7 utilized in the medicinal research and treatment for cancer and could strongly associated to the tumor existence [8]. According to several studies, IL-37 may be employed to suppress the immune system. Additionally, IL-37, which plays a significant regulatory role, has been connected to a number of inflammatory,

autoimmune, and malignant disorders ,by inhibiting the release of pro-inflammatory chemokine's , also the IL-37d encodes the 12-β-strand-which comprises the exons, while IL-37b encodes a transcript containing exons 1 and 2. [9]. Individuals with thalassemia experience the immune system strain as a result of being exposed to different antigens or foreign proteins via blood transfusions. Because of this exposure, they are more vulnerable to infections [10] . Immunological distress can also result from iron excess, a prevalent issue in thalassemia. When a thalassemia patient has a splenectomy, it can change these immune system condition [11]. Understanding the disease pathways requires an understanding of peripheral blood mononuclear cells (PBMCs), which are now fundamental in express the molecular processes that underlie a number of illnesses, by helps with diagnosis and treatment. Nevertheless, a small number of prior investigations have shown changes in immune cells [12]. To fill this knowledge it's important to investigate the gene expression patterns and the role of PBMCs in patients with thalassemia in this study [13]. Mutations and/or deletions in the α-globin or β-globin genes could leading to the hereditary hemoglobin [14] .The thalassemia are linked to β-globin gene mutations due to the decreased in the synthesis of β-globin , as wheel as the defects in hemoglobin could causing the thalassemia disease [15] . An electrophoresis techniques considered an important technique to conduct the hemoglobin abnormalities when a patient is suspected of having thalassemia or hemoglobinopathy based on abnormal complete blood count. The purpose of this study is to clarify the immunological and genetic pattern that could influence the severity of thalassemia [16].

2. Materials and Methods

2.1 The thalassemia patients

About 160 subjects concluded 80 patients with beta thalassemia major (45 female and 35 male) who visited the genetic hematology center at the Feminine and Children Ibn Al-Baladi hospital in Baghdad between October 2024 and February 2025 compared with 80 healthy controls. This study did not include the individuals with Hepatitis B and C, or patients who had had their spleen removed.

2.2. Isolation of PBMC

Each participant had 5 ml of peripheral blood drawn in EDTA vials for additional processing. A modified approach was used to separate (PBMCs) utilizing density gradient separation. The procedure was centrifuged at 350g for 30. PBMC-containing distinct layers were created and gathered. Following two PBS washes, these cells were ready for genetic analysis [17].

2.3 RNA extraction and sequencing

Using about one milliliter of Trizol reagent from the RNA kit (ThermoFisher, Cat. No. 12183555), total RNA was extracted from PBMCs. The absorbance of each RNA sample was measured at 260 nm and were examined using ethidium bromide staining and gel electrophoresis to make sure that the RNA was not broken down, approximately 1 g were used for the ethidium bromide staining to visualize the standard RNA size markers [18] .

2.4 Estimation the levels of IL-7 and IL-37

Using enzyme-linked immune sorbent assay (ELISA) kits from Bioassay Technology Lab., China, the level of (IL-7, IL37) was determined in accordance with the manufacturer's instructions [19] .

4. Resulta and Disscussion

The laboratory and clinical tests may yield the first diagnosis of β-thalassemias. A comprehensive laboratory analysis includes hemoglobin electrophoresis, determining the full blood count, evaluating the morphology and abnormality of red blood cells (RBCs) (Table 1).

Table 1 The laboratory and demographic details of thalassemia patients

Parameters	Number
Patients ,	n 80
Female sex,	n 35 (43.75)
Male sex,	n 45 (55)
Age, years	33 (1–57)
Erythrocytes × 106/μL	5.07 (5.1–7.64)
Hemoglobin in g/dL	11.5 (10–15)
MCV, fL	61 (58–76)
MCH, pg	21 (18–24.8)
MCHC, g/dL	29 (28–33.3)
Reticulocyte count, × 103/μL	95 (21.5–147)
RDW, %	14 (10–27)
HbA2, %	4.5 (3.8–9.1)
HbF, %	1.7 (0.5–4.6)

The distribution of thalassemia major patients was greater in males than in females as shown in (Table 2).

Table 2. Distribution of patients with thalassemia major according to gender

	Group	Patients	Negative control
Gender	Male	45 (56.25) %	31(38.75)%
	Female	35 (43.75)%	49(61.25)%
Total	80		

The results of the current study differed from the results of Javed and Jatha (2020), as the incidence of thalassemia major was higher in females than in males. Our results also differed from the researcher Ali *et al* .,(2023) who found that the incidence rate in males was higher than in females. However, the current results were consistent with the study of Al-Ali and Faraj (2016), which showed that males are significantly more affected than females [20].

A thousand-fold magnification of light microscopy has been used to determine.

the number of cells ,all of the patients in our study group displayed the morphological and numerical abnormalities, including target cells, anisocytosis, and poikilocytosis. All of the slides we examined had anisocytosis and poikilocytosis, which have been identified as common morphologic characteristics in thalassemia carriers. (Table 3) [21].

Table 3: Shows the prevalence of erythrocyte morphological defects in 80 thalassemia patients.

Cell with abnormal morphology	Prevalence, n (%)	Cells/20 HPF, median (range)
Anlsocytosis	9 (11.2)	n.a.
Polkllocytosis	8 (10)	n.a.
Target cells	11(13.7)	42 (4–279)
Ovalocytes	13 (16.2)	13 (0–85)
Dacryocytes	12 (15)	6 (0–23)
Elliptocytes	11 (13.7)	7 (0–46)
punctate basophilia	7(8.7)	7 (0–101)
Irregularly contracted cells	9(11.2)	6 (0–102)
Erythroblasts	0	0

Thalassemia is a hereditary condition that can be lethal in certain individuals. Globally, a disproportionately high number of persons are afflicted by the illness. Our findings are generally consistent with a study conducted by the researcher (Al-Musawie *et al*.,2022), who looked into the most important genetic mutations [22,23]. RNase mapping was carried out in accordance with Melton *et al.* [24] (figure 3) .

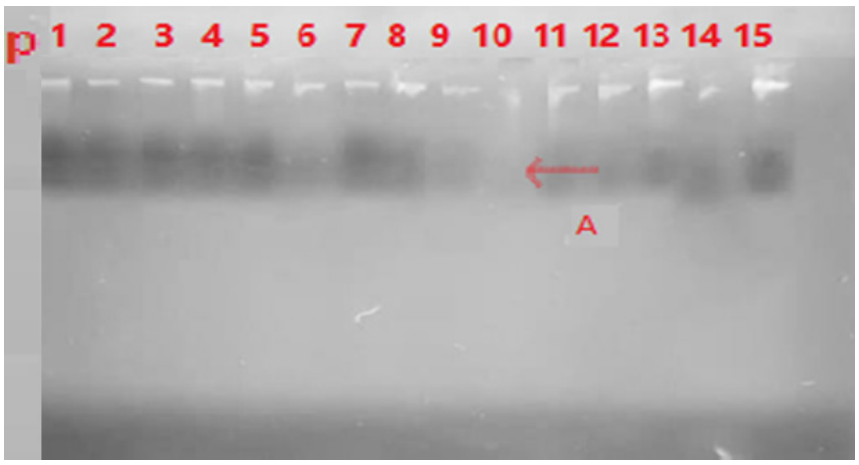

Figure 3 RNase, (A) point shift mutation in patients 10 .

Particularly those that appeared more than once in the examined samples, as well as the contributing factors. Numerous mutations were found to be recurrent in the examined samples. Among these mutations were translocation, deletion, and substitution mutations. This implies that the gene contains weak spots that are prone to mutations, which alters the type of protein that is made [25]. The majority of the unique variants found were located in the intron 2 area, which is significant because intron mutations are known to significantly affect the mRNA stability, RNA splicing, and beta globin gene expression [26].

According to this study, the levels of IL-7 in thalassemia patients were significantly higher (231.34 ±5.11) pg/ml than those of the control group (181.69 ±3.77) pg/ml , and their levels for IL-37 were significantly higher (120.65±2.04) pg/ml compared with the control group (92.86±1.09) pq/ml (Table 4) (figure 4).

Table 2: Comparison between control and patients in IL7 and IL-37

parameter	Patients	controls
IL-7	231.34 ±5.11 pg/ml	181.69 ±3.77 pg/ml
IL-37	120.65 ±2.04 pg/ml	92.86 ±1.09 pg/ml

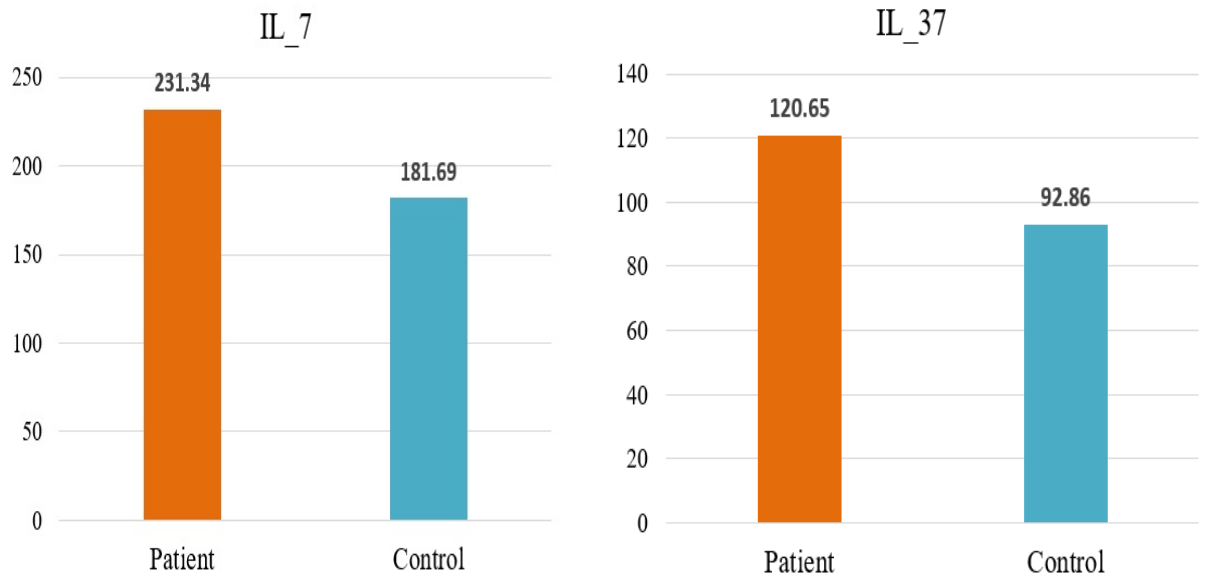

Figure 4 The differences in IL-7 and IL-37 levels between thalassemia patients and controls .

The hematopoietic growth factor IL-7, which influences both T and B cells, was mostly produced by the non-hematopoietic cells and increased the activation of macrophage and monocyte [27]. IL-7 which has six exons and is found on chromosome 8Q12–13, may improve the immunity against viral infections and immunotherapy for cancer treatment, however some studies conducted that the t IL-7 doesn't had important role in the cellular immunological in beta-thalassemia [28].It has been demonstrated that IL-37 can lower the activity of both innate and specific immune responses and suppress the production of inflammatory cytokines in a variety of diseases [29] . While some research suggests that IL-37 inhibits a variety of functions, such the antigen-presenting cells, the activation of macrophage, cytokine generation, and the proliferation of T cell, our investigation found a substantial difference in IL-7 and IL-37 levels between patients and controls [30] . Although IL-37 expression was low in control human tissue, it was found in a large amount of human tissue, including the lymph nodes, ,thymus ,bone marrow, testis ,placenta and uterus. IL-37 inhibits the activity of TLR since it has been strongly linked to both hematological disorder, it was significantly elevated in the current study [31].

Conclusion

The level of IL-7 and IL-37 was significantly higher in thalassemia patients than in healthy controls. The information presented here suggests that the erythropoiesis of patients with thalassemia major is influenced by the generation of IL-7 and IL-37. Therefore, immune-modulating medications may be investigated as substitute treatment choices for thalassemia, as well as the importance of thalassemia mutation profiling.

References

1- Elalfy M, Farid, M, Labib J and RezkAllah H 2014. Quality of life of Egyptian b-thalassemia major children and adolescents, The Egyptian Journal of Haematology 39(4): 222-226.

2- Mustafa I, Firdous N, Shebl F, Shi Z, Saeed M, Zahir Z et al 2020. Genetic epidemiology of beta-thalassemia in the Maldives: 23 years of a beta-thalassemia screening program. Genetics 30: 741 .

3- De Sanctis V, Kattamis C, Canatan D, Soliman A, Elsedfy H, Karimi M et al 2017. β-thalassemia distribution in the old world: An ancient disease seen from a historical standpoint. Mediterranean Journal of Hematology and Infectious Diseases DOI: 10.4084/MJHID.2017.018 .

4- Ricerca, B.M, Di Girolamo, A, Rund, Infections in thalassemia and hemoglobinopathies: focus on therapy-related complications, Mediterr J Hematol Infect Dis 1 (1) (2009) e200 9028.

5- Sinniah D, Yadav M, Elevated IgG and decreased complement component C3 and factor B in a-thalassemia major, Acta Paediatr. Scand. 70 (1981) 547–551.

6- Piyajaroenkij T, Tantiworawit A, Khikhuntod J, et al., Alteration of monocyte subsets and their functions in thalassemia Patients, Int. J. Hematol. 117 (2023) 188–197.

7- Pattanapanyasat K, Thepthai C, Lamchiagdhase P, et al.Lymphocyte subsets and specific T-cell immune response in thalassemia; Communications in Clinical Cytometry2000; 42:11–17.

8- Yang, Y. and Li, D. Z. (2009). A survey of pregnancies with Hb Bart's disease in Mainland China. Hemoglobin, 33(2), 132-136.

9- Yang, Z. et al. Delayed globin synthesis leads to excess heme and the macrocytic anemia of Diamond Blackfan anemia and del(5q) myelodysplastic syndrome. Sci. Transl. Med. 8, 338ra67 (2016).

10 - Thuret, I., Ruggeri, A., Angelucci, E. & Chabannon, C. Hurdles to the Adoption of Gene Therapy as a Curative Option for Transfusion- Dependent Thalassemia. Stem Cells Transl. Med. 11, 407–414 (2022).

11- Kumar, S.; McDonnell, P. C.; Lehr, R.; Tierney, L.; Tzimas, M. N.; Griswold, D. E., et al. (2000). Identification and initial characterization of four novel members of the interleukin-1 family. Journal of Biological Chemistry, 275(14), 10308-10314.

12- Dunn, E.; Sims, J. E.; Nicklin, M. J. and O'Neill, L. A. (2001). Annotating genes with potential roles in the immune system: six new members of the IL-1 family. Trends in Immunology, 22(10), 533-536 .

13- Cannova, J.; Breslin, P. and Zhang, J. (2015) Toll-like receptor sig-

naling in hematopoietic homeostasis and the pathogenesis of hematologic diseases. Frontiers of Medicine, 9(3), 288–303 .

14- Donath, M. Y. and Shoelson, S. E. (2011).Type 2 diabetes as an inflammatory disease.Nature Reviews Immunology, 11(2), 98-107 .

15- Mahmoud, H. M.; Shoeib, A. A.; Abd El Ghany, S. M.; Reda, M. M. and Ragab, I. A.(2015). Study of alpha hemoglobin stabilizing protein expression in patients with β thalassemia and sickle cell anemia and its impact on clinical severity. Blood Cells,Molecules and Diseases, 55(4), 358–362.

16- Theodorou, A. et al. Proteomic Studies for the Investigation of γ- Globin Induction by Decitabine in Human Primary Erythroid Progenitor Cultures. J. Clin. Med. 9, 134 (2020).

17- Li, N. et al. Plasma proteome profiling combined with clinical and genetic features reveals the pathophysiological characteristics of β-thalassemia. iScience 25, 104091 (2022).

18- Bailey, H. J. et al.Humanaminolevulinate synthase structure reveals a eukaryotic-specific autoinhibitory loop regulating substrate binding and product release. Nat. Commun. 11, 2813 (2020).

19-B.M. Ricerca, A. Di Girolamo, D. Rund, Infections in thalassemia and hemoglobinopathies: focus on therapy-related complications, Mediterr J Hematol Infect Dis 1 (1) (2009) e2009028, https://doi.org/10.4084/mjhid.2009.028.

20-J.K. Kao, S.C. Wang, L.W. Ho, et al., M2-like polarization of THP-1 monocyte-derived macrophages under chronic iron overload, Ann. Hematol. 99 (2020) 431–441, https://doi.org/10.1007/s00277-020-03916-8.

21- Ferguson, D. C. J. et al. Characterization and evolutionary origin of novel C(2)H(2) zinc finger protein (ZNF648) required for both erythroid and megakaryocyte differentiation in humans. Haematologica 106, 2859–2873 (2021).

22- Piyajaroenkij T,. Tantiworawit A,. Khikhuntod J, et al., Alteration of monocyte subsets and their functions in thalassemia Patients, Int. J. Hematol. 117 (2023) 188–197, https://doi.org/10.1007/s12185-022-03484-9.

23-Pattanapanyasat K, Thepthai C, Lamchiagdhase P, et al.Lymphocyte subsets and specific T-cell immune

response in thalassemia; Communications in Clinical Cytometry2000; 42:11–17 ; doi.org/10.1002/(SICI)1097-0320(20000215)42:1<11::AID-CYTO3>3.0.CO;2–1.

24- Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T.,Zinn, K. & Green, M. R. (1984) Nucleic Acids Res. 18, 7035-7056.

25- Rivella, S. Iron metabolism under conditions of ineffective erythropoiesis in β-thalassemia. Blood 133, 51–58 (2019).

26- Lechauve, C. et al. The autophagy-activating kinase ULK1 mediates clearance of free α-globin in β-thalassemia. Sci. Transl. Med. 11,eaav4881 (2019).

27- Maia de Oliveira da Silva, J. P. et al. Global gene expression reveals an increase of HMGB1 and APEX1 proteins and their involvement in oxidative stress, apoptosis and inflammation pathways among beta-thalassaemia intermedia and major phenotypes. Br. J. Haematol.186, 608–619 (2019).

28-Psatha, N. et al. Disruption of the BCL11A Erythroid Enhancer Reactivates Fetal Hemoglobin in Erythroid Cells of Patients with β-Thalassemia Major. Mol. Ther. Methods Clin. Dev. 10, 313–326 (2018).

29- Paix, A. et al. Precision genome editing using synthesis-dependent repair of Cas9-induced DNA breaks. Proc. Natl Acad. Sci. USA. 114,E10745–E10754 (2017).

30- Pallis F.R, Conran N, Fertrin Y.K, Olalla-Saad T.S, Costa F.F, Penteado F.F.C. Altered functional properties of eosinophils in sickle cell anemia and effects of Hydroxyurea therapy, Blood 116 (21) (2010) 2656, https://doi.org/10.1182/blood.V116.21.2656.2656.

31- McGrath M.F, Francis A, Fatovich D.M., et al., Genes involved in platelet aggregation and activation are downregulated during acute anaphylaxis in humans, Clin Transl Immunology 11 (12) (2022) e1435, https://doi.org/10.1002/cti2.1435.