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Evaluation of TLR-3, TLR4, IL-7, and IL37 Immunological Markers in β-Thalassemia Major Iraqi Patients

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Abstract: β-thalassemia major is a genetic disorder of hemoglobin production that results in a diminished rate of synthesis of one or more of the globin chains causing variable degrees of anemia. This study was conducted on 90 β-thalassemia patients and 60 healthy as a control group. Blood samples were obtained from Wasit Center for Hereditary Anemia through the period from August 2020 to January 2021. The study was conducted to evaluate the serum immunological markers (TLR-3, TLR4) and (IL-7, IL37) in Iraqi β-thalassemia patients. The study was approved by the council of the Institute of Genetic Engineering and Biotechnology for the Postgraduate Studies / University of Baghdad. Human TLR-3, TLR-4, IL-7, and IL-37 concentrations were estimated by ELISA. Results appeared that there was a significant increase in the level of TLR-3 and TLR-4 in the serum of β-thalassemia patients as well as a significant increase in the level of IL-7 in β-thalassemia patients (221.34±6.11 pg/ml) compared with the control (171.96±4.77 pg/ml). Whereas a non-significant difference in the level of IL-37 was recorded in patients and the control group. This study suggested the presence of an imbalanced immune condition involving inflammation and immunosuppression in thalassemia patients.

Keywords: β-Thalassemia, toll-like receptors, interleukins, ELISA, Iraq

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Introduction

Beta (β)-Thalassemia is an inherited autosomal recessive disease that causes varying degrees of anemia (1). The molecular defects are caused by point mutations or small deletions that reduce or eliminate -globin chain synthesis. The unmatched -Hb is toxic to itself and other cellular components, resulting in inefficient erythropoiesis and hemolysis across the world (2). Cytokines play key roles in the control of hemopoiesis and immunity. These are biologically active molecules that are mainly produced by immune-competent cells and regulate immune response, inflammation, and hemopoiesis. Haemopoiesis is controlled by at least 30 known cytokines, some of these, such as erythropoietin is present at all times, whereas others such as IL37 and IL-7 are produced in response to specific stimuli (3). Interleukin 7 has recently been discovered as a 25kDa protein, a product of a cell line originating from the bone marrow that facilitates the in vitro development of precursors of B-cells interleukin (4). A study (5) reveals that IL-7 mainly promotes the development and does not help the separation of precursors from B cells. IL-7 is also referred to as pre-B-cell or lymphopoietin growth factor 1. (6). Interleukin-37 (IL-37) was first detected and identified by computer sequence analysis by (7) and named IL-1H4. In 2010, this precursor peptide was

identified by (8) to be the seventh IL-1 cytokine family, the so-called IL-1F7. Ding et al. (9) observed that the immune response could be silenced and called IL-37. IL-37 has been detected in a range of inflammatory, autoimmune disorders, and tumors that play a significant regulative function (10).

Toll-like receptors (TLRs), which are found in innate immune cells, are essential mediators of rapid inflammatory responses and appropriate T-cell activation in response to infection and tissue damage. Accumulating evidence suggests that TLR signaling is involved in normal hematopoiesis and specific hematologic pathologies. Particular TLRs and their downstream signaling mediators are expressed not only in terminally differentiated innate immune cells but also early hematopoietic progenitors (11).

Activation of common signaling pathways by TLRs results in the production of various cytokines including tumor necrosis factor-alpha (TNF-α), IL-1β, IL-6, and IL-12 as well as response elements from alternate pathways that prevent a microbial attack. (12) Serum IL-7, IL-37 TLR3and TLR4 concentrations in β-thalassemia patients were measured to determine whether they are useful for a better definition of immunological and erythropoietic alteration.

Material and Methods

Study Timing and Setting:

This study was conducted during the period from August 2020 to January 2021.All the study experiments were performed at the Institute of Genetic Engineering and Biotechnology for the Postgraduate Studies / University of Baghdad as well as the laboratories of Wasit Centre for Heredity Anemia.

Ethical approval and participants consent

This study was approved by the council of the Institute of Genetic Engineering and Biotechnology / the University of Baghdad. Signed written consent was taken from each individual participating in the study.

Sample size, and selection criteria

The study was designed to be a prospective study. The samples selected included β-thalassemia major patients from Wasit Centre for Hereditary Anemia diagnosed as β-thalassemia major.

The selected ninety β-thalassemia major patients diagnosed by Hb electrophoresis, complete blood count had been diagnosed by the center's physicians and sixty healthy individuals were used as control.

Excluded Criteria

Patients group received therapy for thalassemia were excluded in this study, as well as patients with Hepatitis B and C and splenectomized patients were also excluded.

Five ml of peripheral venous blood samples using non-heparinized test tubes from the left capital veins were collected from each participant and healthy control. The serum was separated by centrifugation at 300 rpm for ten minutes, and then it was converted into Eppendroff tubes and kept at -20°c.

The level of (IL-7, IL37) and (TL-3, TL4) was estimated using enzyme-linked immune sorbent assay (ELISA) kits from Bioassay Technology Lab., China according to manufacturers' instructions.

Statistical Analysis

The Statistical Analysis System-SAS (13) program was used to detect the effect of different factors in study parameters. The least significant difference (LSD) test (Analysis of Variation-ANOVA) was used to significantly compare between means and the Chi-square test was used to significantly compare between percentages (0.01 probability) in this study.

Results and Discussion

Subject Data

A total of 150 blood samples (90) patient's samples and 60 as control) were collected.

Serological Study

Determination of the concentration of TLR3 and TLR4 by ELISA

This study showed a significant increase in the level of TLR-3 in β-thalassemia patients (7.07±0.25) pg/ml comparison with control (4.29±0.17) pg/ml also the results of TL-4 appeared significant increase in the level of TL-4 for patients (5.11±0.23) pg/ml when it compared with the control group (3.01±0.17) pg/ml as shown in Figure (1) and Figure (2) and Table (1).

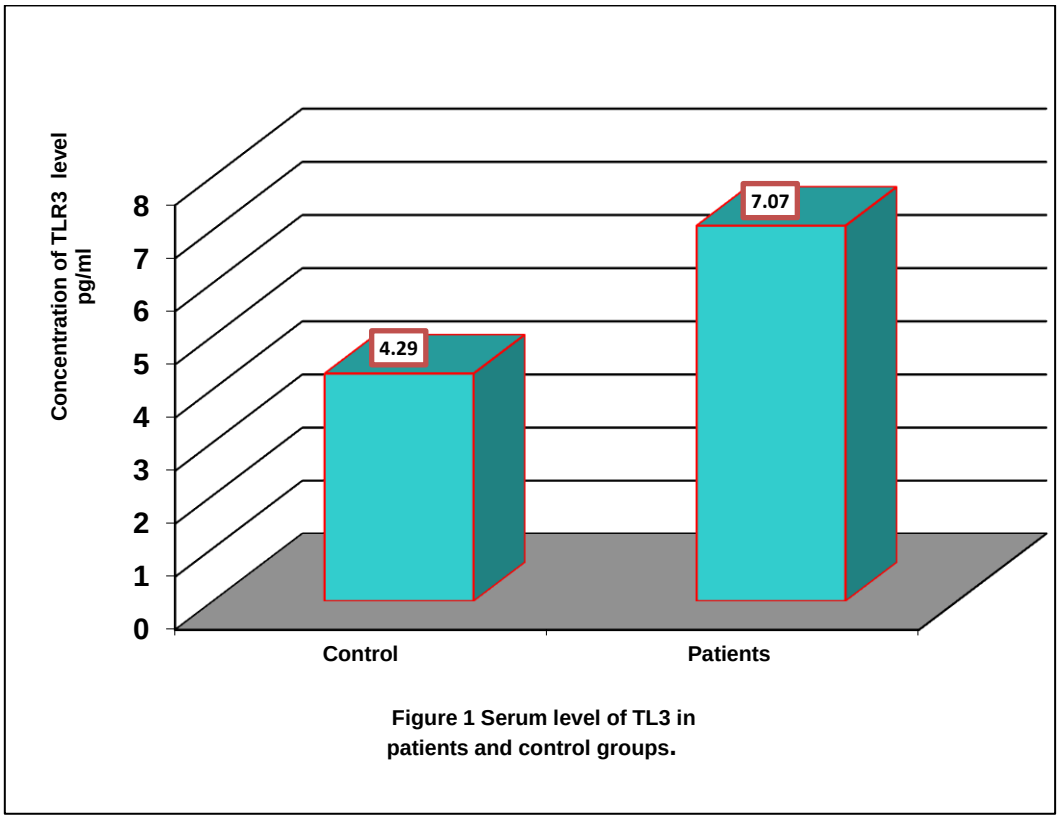

Figure 1 Serum level of TL3 in patients and control groups.

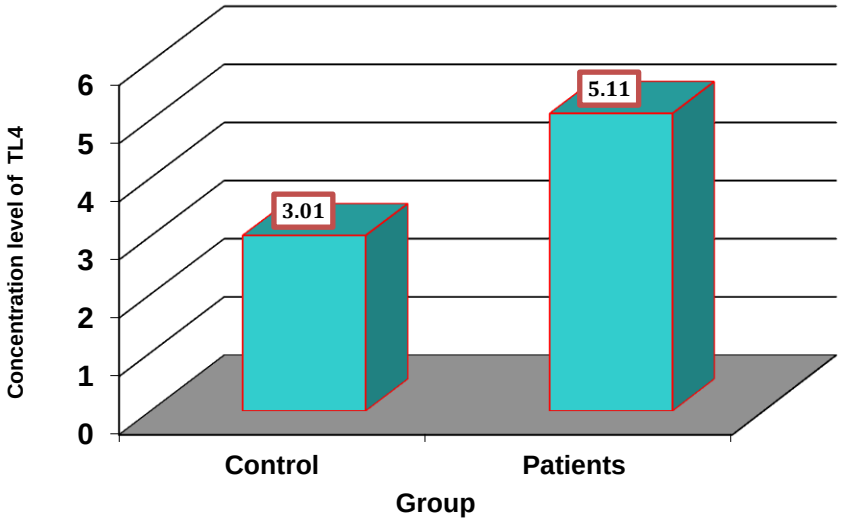

Figure 2. Serum level of TL4 in control and patients groups

Table 1: Comparison between control and patients in TL3 and TL4

Group	Mean ± SE	
	ELISA TL4	ELISA TL3
Control	3.01 ±0.17	4.29 ±0.19
Patients	5.11 ±0.23	7.07 ±0.25
T-test	0.625 **	0.693 **
P-value	0.0001	0.0001
** (P≤0.01).		

TLR stimulation induces the production and gene activation of diverse cytokines including both pro-inflammatory and anti-inflammatory cytokines depending on cell type and environment (14).

Toll-like meaning amazing in Germanal–like receptors represent ancient host defense pathways.

TLR family and a key receptor for the recognition of gram-negative bacteria, fungi, viruses (15, 16).

Up-regulation of TLR -4 levels has been associated with an increase in inflammatory response (17).

TLR is a class of receptor involved in nonspecific immunity, acting as a bridge to link the nonspecific and specific immune response (18).

When microorganisms infiltrate physical barriers such as skin and mucous membrane, TLR binds them and stimulates immune cell response (19, 20).

TLR involved various immune system diseases including Rheumatoid arthritis, osteoarthritis, and autoimmune myositis (21, 22).

TLR-4 is a ligand of lipopolysaccharide hyaluronic acid and the heat shock portion can promote the production of TNF-α IFN, IL-12, and more pro-inflammatory factors including inflammatory damage (23).

The current study showed a significant increase in TLR-3 and TLR-4 levels in the serum of patients than control but Zhang *et al*. (2019) suggest increased TLRs expression and suppressed the expression of TLR-4 in Kylosig spondylitis the disagreement due to the difference in disease.

TLR is present on macrophages, dendritic cells, neutrophils, and mast cells as well as on cells of the adaptive immune system T and B cells. These cells have characteristic TLR expression patterns which can direct them to specific behavior (25, 26).

The TLR signaling leads to the activation of self-reactive T or B cells. The activation of such self-reactive cells may be due to the presence of some dangerous signals, derived from microorganisms that break immunological tolerance of the host and further lead to the development of autoimmune disease, TLR can also activate the antigen-presenting cells macrophages (27), in the current study observe significant increase the TLR3 and TLR4 in the serum of β-thalassemia major function because more susceptible to infection by several microorganisms.

TLR plays a critical role in inducing the expression of pro-inflammatory cytokines gene, thereby activating both innate and adaptive immune response (28).

TLR 3 is expressed in the B cells and stimulated macrophage, but TLR cannot recognize endogenous RNA except those released from necrotic.

As a result of tissue inflammation (29), TLR overexpression might suggest that specifically, B cells were reacting to latent microbial agents during remission (30).

TLR3 expression was highly variable between individuals and determined the response of the immune system to chronic viral infection TLR3 induce production of IFN (31).

The results of the current study showed a significant increase in the level of TLR3 and TLR4 in the serum of patients compared with the control group may be the β-thalassemia major patients more susceptible to infection.

Determination of the concentration of IL-7 by ELISA

IL-7 was secreted by stromal cells of bone marrow and acts predominately on T and B lymphocytes pronation, its function as a growth and differentiation factor for both T and B cells precursor. IL7 was produced in response to specific stimuli (32).

The results in our study have disagreed with (33) in Turkey because they observed a non-significant difference in the concentration of IL-7 between thalassemia patients and control while the current study showed a significant increase in the level of IL-7 in the serum of the β-thalassemia and control this difference in the results of IL-7 may be due to differences in the race of the patients, environmental factors, and nutritional state.

Bolotin (34) observed a significant increase in the level of IL 7 in bone marrow transplant recipients in the U.S.A. IL-7 can induce the proliferation of T cells (35).

IL-7 was a hematopoietic growth factor that modulates both T and B cells (36) production of IL-7 was primarily from the non-hematopoietic cell (37, 38) and up-regulated T-cell dependent activation of monocytes macrophages (36, 39).

IL-7 was located on chromosome 8Q12-13 and contains 6 exons (40) and IL-7 enhances immunity to viral infection, (41) and immunotherapy for cancer treatment mice deficient in IL-7 suffer from lymphopenia (42, 43).

Determination of the concentration of IL-37 by ELISA

The results in the current study appear significant increase in the level of IL-7 in β-thalassemia patients (221. 34±6.11) pq/ml when compared with

control (171.69±4.77) pq/ml whereas the non-significant difference in the level of IL-37 in patients (96.65±3.04) pq/ml and (94.86±2.09) pq/ml in the serum of the control group as presented in (Table 2).

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

Group	Mean ± SE	
	ELISA IL-7	ELISA IL-37
Control	171.69 ±4.77	94.86 ±2.09
Patients	221.34 ±6.11	96.65 ±3.04
T-test	16.69 **	8.10 NS
P-value	0.0001	0.661
** (P≤0.01).		

Interleukin -37 a member of the interleukin family, was an anti-inflammatory cytokine produced by immune cells and suppresses the production of inflammatory cytokines in several types of diseases, it has been shown that IL-37 was capable of reducing the activity of both innate and specific immune responses (48, 9).

In our study, there was a non-significant difference in the level of IL-37 in patients and control, whereas (49) observed IL-37 was elevated in the serum of hepatitis B patients, but (50) suggested that IL-37 inhibit various functions such as antigen-presenting cells, macrophage activation (51) and cytokine production and inhibit antigen-specific T cell proliferation (52). In addition, IL-37 has been demonstrated to be expressed by immune-suppressive regulatory T-cells (53). IL-37 was detectable in much human tissue but the expression was low in control human tissue (9, 54), IL-37 expression had been detectable in liver, lung, thymus, bone marrow, lymph nodes, placenta, testis, uteri, and tumor tissue (55). IL-37 suppresses the function of TLR (9, 56) and in the current study, the IL-37 was non-significant increased whereas TLR-3 and TLR-4 significant increase and these results were agreed with our study Il-37 inhibit the activation of Th2/Th2 (57). IL-37 was excessively expressed in the synovial tissues of patients with which active Rheumatic arthritis (58, 59), and the IL-37 was significantly higher in SLE patients than control (53) whereas decreased in asthmatic patients childhood (44). IL-37 may be closely related to obesity and insulin resistance (45, 46) whereas the results of the current study showed a non-significant difference in the level of IL-37 for β-thalassemia patients and the control group. Immune modulation in thalassemia patients with regional specific variation was related to variation in treatment modulation (47).

Conclusion

In our study, although no significant difference in serum IL-37 concentrations between thalassemia patients and healthy controls was found, the level of IL-7, TLR-3, and TLR-4 was significantly higher in the thalassemia patients compared with the healthy control. As a general, hemopoietic growth factor production is a cascade reaction regulated in such a way as to permit flexible and integrated responses to diverse hemopoietic stresses. Increasing levels of IL-7 and TLR-3,4 appear to be associated with increased hemopoiesis**.** According to the data reported here, the involvement of IL-7and TLR3,4 production in erythropoiesis of thalassemia major patients has been suggested.

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