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# REGULATORY ROLE OF INTERLEUKIN 10 IN IRAQI THALASSEMIC PATIENTS INFECTED WITH TOXOPLASMOSIS

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**Abstract**

Toxoplasmosis is a parasitic disease caused by Toxoplasma gondii which it is an obligatory intracellular pathogen protozoan that infects mammalian hosts. Thalassemia is a group of inherited microcytic, hemolytic anemia that are characterized by abnormal hemoglobin production. Interleukin 10 is an anti-inflammatory cytokine keeps the immune systems reaction in check and promotes infection clearance with the least amount of host damaging effects. The current study was designed to detect this regulatory role of Interleukin 10 in thalassemic patients infected with toxoplasmosis. In the present study, 165 cases of thalassemic patients and 80 cases of healthy controls with age range from 2-45 years, were collected between March to June 2022 from Al- Karma Teaching Hospital in Baghdad, Iraq. Anti-Toxoplasma IgM/ IgG antibodies detected by CMIA followed by measuring WBC and lymphocyte concentrations by using cell-DYN ruby hematology analyzer. ELISA method used to demonstrate IL-10 levels. The results of this study revealed that the group of thalassemic patients with toxoplasmosis has highest level of anti-Toxoplasma IgG antibody(60/165) 41.475±9.193 UI/mL, whereas the group of toxoplasmosis control seropositive has level of anti-Toxoplasma IgG antibody (25/80) 35.59±8.336 UI/mL according to CMIA, as well as findings demonstrated that the group of thalassemic patients with toxoplasmosis has the mean of WBC test 8.12±0.136 ($10^9$/L) pursued by the group of the group of healthy control 4.902±0.168 ($10^9$/L) in the same test. Nevertheless, the result in the group of thalassemic patients with toxoplasmosis of lymphocytes was 3.2725 ± 0.023 ($10^9$/L) in compared with the healthy control group was 2.488 ± 0.037($10^9$/L), while all groups were seronegative response for anti-Toxoplasma IgM antibody. Measuring IL-10 levels in samples were done by ELISA method that showed a group of thalassemic Patients with toxoplasmosis have level of IL-10 (3.9560 ± 0.253) pg/ml, while thalassemic patients recorded level also (4.905± 1.166) pg/ml in comparison with control group (2.925 ± 0.261) pg/ml.

**Keywords:** Toxoplasma Gondii, Thalassemia, IgG, IgM, Interleukin-10

## INTRODUCTION

Toxoplasmosis is a disease caused by the intracellular protozoan parasite *Toxoplasma gondii* which it is an opportunistic parasite in immune-compromised persons, it has a worldwide distribution and it is one of the most prevalent infectious agents in Iraq (1, 2, 3). *T. gondii* has 2 life cycles: the sexual way occurs exclusively in the small intestines of cats, whereas the asexual way takes place in infected animals and humans. In humans, infection is usually acquired by consumption and manipulation of raw or undercooked meat and trans placenta method. The diagnosis of *T.gondii* infection in human can be determined by variable immunological and molecular methods (4, 5).

Thalassemia is a wild collection of blood diseases that disrupt the hemoglobin genes and impair erythropoiesis, it is a typically autosomal recessive form of severe anemia that it is caused by an imbalance of two types of protein alpha and beta subunits of hemoglobin. Early onset of anemia because to decreased hemoglobin production need regular blood transfusions to maintain hemoglobin levels (6, 7).

IL-10 a cytokine has a variety of pleiotropic effects on inflammation and immune function. It reduces co-stimulatory molecules and T-helper1 cytokines on macrophages (8, 9). Domination of toxoplasmosis leads to produce large amounts of proinflammatory cytokines. Therefore, IL-10 is essential for initiating the chronic toxoplasmosis infection. It prevents antigen-presenting cells (APCs) from acting in an inflammatory manner by expressing opposing costimulatory molecules (10, 11, 12).

IL-10 has the ability to inhibit macrophages activation and inducing IFN-γ production by *T. gondii* infection enhance the intracellular parasite survival and can lead to immunological suppression, which it is considered an advantageous to both parasite and the host which increase the ability of the parasite to produce T-helper 2 (TH2) cytokines (13,14). The object of this study is detecting the effect of *T.gondii* on immune response by detecting regulatory role of IL-10 in thalassemic patients infected with toxoplasmosis.

## MATERIALS AND METHODS

### 1. Subjects

The study included 165 individuals suffering from thalassemia and 80 individuals have been chosen as a healthy control who attended to the Al-Karma Teaching Hospital in Baghdad, Iraq during the period from March to June 2022 with ages ranging between (2-45) years after the doctor's diagnosis and necessary blood tests to detect thalassemia, serum samples were examined and diagnosed for anti-*Toxoplasma* IgM and IgG antibodies by using CMIA. Five milliliters of venous blood were withdrawn via using a sterile syringe from each patient. Two ml of whole blood was collected in a labeled EDTA tube for leukocytes count and 3 ml was transferred into a fully labeled gel tube to separate the serum by centrifuge at 3000 rpm for 5 minutes and stored at -20 C° until used in CMIA and ELISA detection.

### 2. T. gondii diagnosis

Chemiluminescent microparticles immunoassay (CMIA) was performed for the detection of both anti –*Toxoplasma* IgG/IgM antibodies in sera according to the manufacturer's instruction (Architect Toxo IgM/G kit -Abbott GmbH, Germany).

### 3. Estimate of Leukocytes Parameters

Total leukocyte counts measured in anti-coagulated blood samples by using CELL-DYN Ruby Hematology Analyzer system by manufacturer Abbott.

### 4. Determination of IL-10 level

Circulating serum levels of IL-10 were measured by sandwich ELISA using commercial kits according to the manufacturer's instructions: ELISA Test Kits provided by (Elabscience USA).

### Statistical Analysis

The Statistical Analysis System- SAS (2018) program (15). was assessed Software Statistical Package for Science, Statistical significance was determined by using L.S.D. test for quantitative dated. Results were expressed as mean±S.D. Chi-square test was used to significant compare between percentage ($P \leq 0.01$, $P \leq 0.05$) probability in this study.

## RESULTS AND DİSCUSSİON

*T. gondii* infection in healthy hosts occasionally manifests symptoms. While, toxoplasmosis in immunocompromised people carries a high risk of pathogenicity (16). Table (1) shown that the group of thalassemic patients with toxoplasmosis has the highest level of anti-*Toxoplasma* IgG antibody (60/165) 41.475±9.193 IU/ml followed by the group of non-thalassemic patients positive control (25/80) 35.59±8.336 IU/mL. while, all the groups were seronegative for anti-*Toxoplasma* IgM antibody.

**Table 1: Concentration of Anti- Toxo IgG / IgM Assay IU/mL in Studied Groups**

| Groups | NO | Mean ± SE UI/mL IgG | Mean ± SE UI/mL IgM |
|---|---|---|---|
| Thalassemic patients with toxoplasmosis | 60 | 41.475±9.193 | 0.211± 0.098 |
| Thalassemic patients | 105 | 0.489±0.084 | 0.073±0.0022 |
| Toxoplasmosis (control positive) | 25 | 35.59±8.336 | 0.103±0.03 |
| Healthy individuals (control negative) | 55 | 0.5616±0.246 | 0.0422±0.004 |
| LSD value | | 13.64 ** | 0.0662 * |
| P-value | | 0.0056 | 0.0278 |
| * (P≤0.05), ** (P≤0.01). , Primary (acute) infection ≥ 0.6. , Secondary (chronic) infection ≥ 3.0 | | | |

The above results similar to the results of Yousef *et al* (17) that demonstrate 30.76% (36/117) of thalassemic patients and 20% (41/205) of the healthy control were seropositive of anti-*Toxoplasma* IgG antibody. Whereas, the same study dissimilar in the anti-Toxoplasma IgM detection which found low rate 1.70% (2/117) and 0.48 (1/205) of thalassemic patients and healthy control respectively were seropositive for the anti-*Toxoplasma* IgM antibody. The majority of patients with sickle cell anemia and those with severe types of thalassemia depend on the blood product infusions to optimize their clinical status as a result of these hemoglobinopathies. Moreover, once serological screening is not required for all microorganisms, there is a danger of infections by viruses, bacteria, and parasites by transfusions (18,19).

Furthermore, table (2) revealed the means titers of WBC in thalassemic patients with toxoplasmosis was 8.12±0.136 in comparison with other studied groups as well as the mean titer of lymphocytes in same group was 3.2725 ± 0.023 in comparison with the other groups.

**Table 2: WBC and Lymphocytes Concentrations in Thalassemic and Non-Thalassemic of Studied Groups**

| Groups | Mean ± SE | |
|---|---|---|
| | WBC | Lymph |
| Thalassemic patients with toxoplasmosis | 8.12±0.136 | 3.2725 ± 0.023 |
| Thalassemic patients | 8.436±0.107 | 3.213 ±0.022 |
| control positive (Infected with toxoplasmosis) | 4.902±0.168 | 3.020 ±0.022 |
| Healthy individuals control negative | 5.508±0.103 | 2.488 ±0.037 |
| LSD value | 2.078 ** | 1.027 * |
| P-value | 0.0001 | 0.047 |
| * (P≤0.05), ** (P≤0.01). | | |
| WBC (3.70-10.1per microliter), Lymphocytes (1.09-2.99 in 1 microliter) | | |

The findings of above results demonstrate that total WBC counts significantly increased in the groups thalassemic patients with/without toxoplasmosis when compared with the control groups, this explained by an increase in the production of certain cytokines such as IL-3 which encourages the precursor cells to develop into white blood cells in the bone marrow (20). The elevation in the count of total WBC in thalassemic patients was illustrated by Roshdy *et al.* (21) that referred to the repeated exposure to various infectious agents during routine blood transfusions and ongoing WBC synthesis for infection agent defenses (22).

Thirty samples of thalassemic patients with and without toxoplasmosis groups as well as 25 samples of non-thalassemic control groups were used to assess the IL-10 levels via Sandwich ELISA method.

The results in table (3) illustrated that the group of thalassemic patients has highest level of IL-10 4.905± 1.166 pg/ml followed by the group of thalassemic patients with toxoplasmosis 3.956±0.253 pg/ml then the group of non-thalassemic positive control 3.717±0.392 pg/ml. However, the healthy control group has the lowest level of the same interleukin 2.925±0.261 pg/ml.

**Table 3**: **levels of IL-10 cytokine (pg/ml) in the sera of the studied groups**

| Groups | NO | Mean ± SE pg/mL |
|---|---|---|
| Thalassemic Patients with toxoplasmosis | 30 | 3.9560 ± 0.253 |
| Thalassemic patients | 30 | 4.905± 1.166 |
| Toxoplasmosis patients control positive | 25 | 3.717 ± 0.392 |
| Healthy individuals control negative | 25 | 2.925 ± 0.261 |
| LSD value | 1.437 * | |
| P-value | 0.0337 | |
| * (P≤0.05). | | |

The above results of Matowicka-Karna *et al* (23) that demonstrate the group of patients infected with toxoplasmosis has the highest level of IL-10 (2.9 ± 0.76 pg/mL) as well as the group of healthy control has 0.2 ± 4.57 pg/mL of the same interleukin.

This study similar to the results of Mahmoud *et al* (24) that found the group of thalassemic patients infected with hepatitis has high in the concentration of IL-10 33.62 ± 7.3 while the group of thalassemic patients has 8.74 ± 2.5 of the same interleukins.

Interleukins are playing a significant role in the body's response to injury. IL-10 is secreted by T helper-2 type cells (Th2) that may inhibit cell-mediated immune effector pathways vital to the host's defense against intracellular infections, different roles of cytokines IL-4, IL-10, IL-12p40, IFN- during toxoplasmosis disease (25,26). *T. gondii* infection has demonstrated that CD4 T-cells are crucial for maintaining role in the effector response of CD8 T-cells. CD4 T-cell exhaustion is related to CD8 T-cell dysfunction (27).

IL-10 counteracts the damaging effects of the inflammatory response which is based on the increase production of TNF-a, IFN and NO related with intestinal multiplication of toxoplasmosis (23). Additionally, Jison *et al.* (28) demonstrated that IL-10 is a cytokine with strong anti-inflammatory activity that inhibits the production of numerous cytokines such as IL-1, IL-6, IL-8, IL-12, TNF-a, and GM-CSF to promote iron uptake and retention in the reticuloendothelial system. conclusion

Present study found higher rates of *T. gondii* seropositivity and highly significant difference in the IL-10 levels in thalassemia patients infected with toxoplasmosis.

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