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Role of interferon-gamma (IFN-γ) in Iraqi thalassemic patients infected with Toxoplasmosis

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Abstract

Background: Toxoplasmosis is caused by the intracellular protozoan parasite known as *Toxoplasma gondii*. This parasite infects all types of endothermic animals, including mammals and birds, and is widespread throughout the world. A widespread form of hereditary anemia and the most common cause of hematologic disorders is Thalassemia. Interferon (IFN) is necessary for cellular immune activation against viral, bacterial, and parasitic infections. This study aims to evaluate the role of interferon-γ (IFN-γ) in modulating immunity in thalassemia patients infected with *T. gondii*.

Methods: Blood samples were collected from the period of March-June, 2022, from 165 thalassemia diagnosed individuals and 80 healthy volunteers at Al-Karama Teaching Hospital, Baghdad, Iraq. The ages of all patients were from 2 to 45 years. Thalassemia was diagnosed by blood tests conducted as per doctor's prescription.

Result: The thalassemic individuals infected with toxoplasmosis showed the highest IgG antibody titers, while the control group with toxoplasmosis ranked second. The thalassemic individuals, with or without toxoplasmosis, also showed significantly higher concentrations of IFN-γ compared to the *T. gondii*-positive control group and healthy subjects.

Conclusion: It is concluded that the highest IFN-γ levels were found in the thalassemic group as compared with other study groups.

Introduction

Toxoplasma gondii is an opportunistic parasite with zoonotic potential, which leads to toxoplasmosis, an infection with broad distribution in the world. In Iraq, the pathogen is very prevalent and can spread through several routes. It is of great medical and veterinary significance because of its direct contribution towards the health of animals and humans. *T. gondii* further serves as an opportunistic pathogen especially in immunocompromised individuals [1-5]. Toxoplasmosis is usually acquired through ingestion of the sporulated oocysts, which are shed from the feline host and can infect food and water. Infection can also result from the consumption of undercooked or contaminated meat that contains tissue cysts. Besides, rapidly dividing tachyzoites can invade and replicate within many tissue and cellular systems. This widespread tissue replication can cause acute infection and great tissue damage in various organs [6].

Toxoplasmosis can cause serious, occasionally deadly, side effects, especially in immunocompromised individuals and pregnant women. These are individuals with HIV/AIDS, cancer, and persons receiving an organ transplant. Those with diabetes are also exposed to increased risk because they have weakened immune systems. To diagnose *T. gondii* infection, healthcare practitioners use both serological examinations and molecular diagnostic methods [7-10].

Thalassemia is an autosomal recessive genetic condition that affects hemoglobin synthesis [11]. α-thalassemia and β-thalassemia are the two dominant types, caused respectively due to mutations of the α-globin and β-globin genes. Less frequent versions of the condition occur due to mutations of other globin genes. When the synthesis of any one of these globin chains is defective, hemoglobin synthesis is also defective, which results in the formation of microcytic (small) and hypochromic (pale) red blood cells [12-14].

Thalassemia is linked to a number of innate immune system disorders that can be seen as early as childhood. Reduced levels of complement, properdin, lysozyme, diminished polymorphonuclear neutrophil phagocytic capacity, disrupted chemotaxis, and altered intracellular metabolic processes are a few of them. Also, multiple blood transfusions may trigger the disruption of chemotaxis and phagocytosis of the monocyte-macrophage system as a result of prolonged immunological activation via foreign proteins [15]. Immune cell characteristics, both quantitative and qualitative, as well as the cytokine profile of innate immunity, are all affected by derangements in thalassemia major (TM). Patients with TM frequently have greater total leukocyte, neutrophil, and lymphocyte counts and a low-grade systemic inflammatory state [16].

An efficacious multifunctional cytokine, interferon-gamma or IFN-γ, plays significant roles in several biological processes which include defense against viral infection, preventing tumor growth, and regulating the immune response. The cytokine is an incredibly potent enhancer of both innate and adaptive immunity. During inflammation, protective immune function is reinforced to clear infection by IFN-γ. It is also an inhibitory factor, which prevents over-activation of the immune response and consequent tissue damage [17,18]. This study was done to evaluate the role of IFN-γ in modulating immunity or immune response in thalassemic patients infected with toxoplasmosis.

Methods

Subjects and Samples

The study recruited 165 thalassemia patients and 80 healthy controls, and age range among participants varied from 2 to 45 years (average age: 15.39 ± 0.63 years). Hematological investigations were conducted to check thalassemia status in all the subjects.

Venous blood samples (5 mL each) of all participants were collected for laboratory analysis. A total of 3 mL of each of these samples were immediately put into gel-activation tubes and kept at room temperature (20-25°C) and left to clot over the course of 15 minutes. Centrifugation after that at 3000 revolutions per minute for 10 minutes provided serum fractions.

The serum samples were isolated and analyzed extensively for *Toxoplasma gondii*-specific IgM and IgG immunoglobulins as well as for the level of interferon-gamma (IFN-γ) through the use of the sandwich enzyme-linked immunosorbent assay (ELISA) technique.

T. gondii diagnosis

The Architect Toxo IgM/G kit (Abbott GmbH, Germany) was used to measure the levels of IgM/IgG according to the procedure specified by the manufacturer.

Estimation of IFN-γ levels

Sandwich ELISA technique was employed for measuring the levels of IFN-γ as per the manufacturer's directions for Human IFN-γ kit (Elabscience, USA).

Statistical Analysis

The data were analyzed statistically with SAS software (2018) to assess the effect of different variables on study variables [19]. Comparisons of means were made with Analysis of Variance, with post-hoc testing through the Least Significant Difference (LSD) method. For the purposes of comparing categorical data, the chi-square

Group name	Total number of samples	IgM	IgG	P-value
Individuals with Thalassemia	165	0.0%	36.36%	0.0001**
		(0)	(60)	
Non-thalassemia control	80	0.0%	31.25%	0.0001**
		(0)	(25)	
P-value		0.0001**	NS	-

Table 1: Anti-T. gondii antibodies (IgG/IgM) percentage in patients with and without thalassemia.
**(P ≤ 0.01) highly significant

Groups	Number & Percentages	Mean ± SE IU/mL (IgG)	Mean ± SE IU/mL (IgM)
Toxoplasmosis-carrying thalassemic patients	60 36.40%	41.475 ± 9.193	0.211 ± 0.098
Thalassemic patients	105 63.60%	0.489 ± 0.084	0.073 ± 0.0022
Positive control (Infected with toxoplasmosis)	25 31.25%	35.59 ± 8.336	0.103 ± 0.03
Healthy individuals (negative control)	55 68.75%	0.5616 ± 0.246	0.0422 ± 0.004
LSD value		0.0662*	13.64**
P-value		0.0278	0.0056

Table 2: Percentages and levels of anti-Toxoplasma IgG and IgM in the thalassemic and non-thalassemic groups.
SE = Standard error

Groups	Mean ± SE	
	WBC	Lymphocyte
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

Table 3: WBC and lymphocyte concentrations in studied groups with and without thalassemia.
SE = Standard error

analysis was utilized to examine statistically significant differences in findings, with significance at the level of P ⩽ 0.01 and P ⩽ 0.05.

Results

Furthermore, Table 1 demonstrated percentages of toxoplasmosis infections and clearly stated that 36.36% or 60 patients out of a total of 165 from the thalassemic group were positive for Toxoplasma-specific antibodies, and 25 patients out of 80, or a percentage of 31.25% of non-thalassemic group patients were positive for the same specific Toxoplasma antibodies. The results showed a significant difference (P ⩽ 0.01) as evident from Table 1. Below is a figure showing the ratio of thalassemic and non-thalassemic patients in their respective groups (Figure 1).

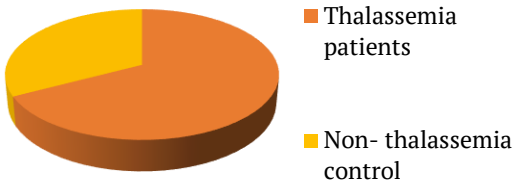

Figure 1: The percentage of patients with and without thalassemia in their respective groups.

T. gondii diagnosis

The group of patients with thalassemia and toxoplasmosis (36.40%) or 60/165 were the most positive for IgG antibodies, which were 41.475 ± 9.193 IU/mL according to Chemiluminescent Microparticle Immuno Assay (CMIA) (Table 2). The group of positive control (31.25%) or 25/80 was also positive for IgG antibodies, but their level was less than the group of toxoplasmosis carrying thalassemia patients, with a level of 35.59 ± 8.336 IU/mL. While the remaining groups lacked the required IgM antibodies.

In addition, Table 3 showed that the mean titer of WBC in thalassemic patients with toxoplasmosis was 8.12 ± 0.136 in comparison with other studied groups, and the mean titer of lymphocytes in thalassemic patients with toxoplasmosis was 3.2725 ± 0.023 in comparison with the other groups.

Assessment of IFN-γ

The results of this study in Table 4 revealed highest level of IFN-γ was recorded in the thalassemic patients' group, with a value of 24.254 ± 3.117 pg/mL, followed by the toxoplasmosis-carrying thalassemic patients group, 24.0216 ± 1.932 pg/mL, in comparison with other study groups.

Groups	No.	Mean ± SE pg/mL
Toxoplasmosis-carrying thalassemic patients	30	24.0216 ± 1.932
Thalassemic patients	30	24.254 ± 3.117
positive control (Infected with toxoplasmosis)	25	15.353 ± 6.412
Healthy individuals (negative control)	25	11.122 ± 1.889
LSD value	5.491**	
P-value	0.0001	

Table 4: IFN-γ levels in sera of the studied groups.
**(P ≤ 0.01)

Discussion

Toxoplasma gondii is commonly known as an incredibly successful parasite, known for three distinguishing features: its worldwide distribution, incredible range of hosts it can infect, and invariably high incidence rates all over the planet. Such harmful characteristics cause toxoplasmosis to be an opportunistic illness that can cause severe damage [20,21]. A study on thalassemic children by El-Tantawy *et al.* (2019) showed that 23.2% or 49 out of 211 children were positive for IgM antibodies, and 53.6% or 113 out of 211 children were positive for IgG antibodies during Toxoplasmosis infection [22]. Alternatively, the occurrence of IgM and IgG antibodies was 5% (5/100) and 18% (18/100) during *T. gondii* infection, respectively, in the group of healthy controls. This study is similar to the current study in detecting Toxoplasma-specific antibody (IgG), where a low percentage of IgG was detected in the positive control group suffering from toxoplasmosis infection, and was different in detecting IgM, where it was detected in the same positive control group.

The present study corresponds to the study of Hanifehpour *et al.* (2019) that showed the group of thalassemic major patients (51.9%) or 122/235 were seropositive for anti-Toxoplasma IgG as compared to the group of healthy individuals (34.8%) or 82/235 [23].

Blood transfusion can be a source of various infections if infected blood is used directly or picked up from unhygienic conditions. Particularly viral or parasitic infections, and the blood is the primary means of transportation [24,25].

The results above show that total white blood cell (WBC) counts significantly increased in the group of thalassemic patients with/without toxoplasmosis when compared to the control groups, which is accounted for by an increase in the production of specific cytokines like IL-3, which encourages the precursor cells to develop into white blood cells in the bone marrow [26]. According to Roshdy *et al.* (2018), who discussed frequent exposure to various infectious agents during normal blood transfusions and ongoing WBC synthesis for infection agent defenses, the elevation in the count of total WBC in thalassemic patients was illustrated [27,28].

In individuals with thalassemia, immunological abnormalities affecting both innate and adaptive immunity are thought to have a significant role in morbidity and mortality. Patients with thalassemia not only have a higher risk of blood-borne infections from multiple transfusions, but they also have an immunological weakness that makes them more susceptible to infections [29].

IFN-γ is crucial for the stimulation of anti-microbial IFN-γ inducible genes, which leads to the clearance of intracellular microbes, making it necessary for the host defense against toxoplasmosis, inducing innate and adaptive type I immune response to intracellular infections due to a powerful inducer of IFN-γ [30]. According to the study of Ismael and Salih (2019), the sera of aborted women infected with toxoplasmosis have a raised level of IFN-γ compared with healthy women, which confirms the role of IFN-γ in the immune response against toxoplasmosis [31]. According to a study by Abdul-Lateef *et al.* (2012), *T. gondii* triggers a cellular immune response that is visible as a statistically significant rise in IFN-γ levels in toxoplasmosis patients compared with the healthy control group [32].

Study of Obeid *et al.* (2018) recorded that the levels of IFN-γ were significantly increased in thalassemic patients by a value of 38.6 ± 10.5 pg/mL as compared to the control group, which had a value of 5.55 ± 1.70 pg/mL [33]. They demonstrate that the elevated IFN-γ levels observed in thalassemic patients may result from the direct effects of iron overload on immune system cells and other damaged body organs which cause activation and release of various cytokines and immune markers regardless of the presence of infections, whereas the increased serum levels of IFN-γ may be due to the immune system being activated as a result of the direct effect of iron on various body organs. A few studies demonstrate that toxoplasmosis infection can change the behavior of chronic infections by increasing levels of hormones linked to behavior [34]. It was made clear by another study that hemodialysis patients had a higher incidence of toxoplasmosis than healthy people [35].

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Author Contributions

Conceptualization, writing of original draft, methodology, data curation: Raghad N. Shihab
Supervision, writing, review, and editing: Israa Kasim Al-Aubaidi

Conflict of Interest

There is no conflict of interest in the publication of this work.

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