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Original Article

Effects of *Toxoplasma gondii* on Levels of Interleukin-5 in Parkinson's Patients

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Abstract

Parkinson's disease (PD) is a psychiatric neurological infection of the focal sensory system and is accepted as a multifactorial disease. Chronic Toxoplasmosis is sometimes associated with the proliferation of bradyzoites in the nervous system. The measurement of interleukin-5 (IL-5) as an inflammatory mediator in patients with PD and Toxoplasmosis infection may be helpful in determining the correlation between these diseases. In the present study, 80 examples were collected, including 35 patients diagnosed with idiopathic PD and 45 samples from healthy people from Najaf, Babylon, and Baghdad provinces, Iraq. After measuring the immunoglobulin G (IgG) antibody of *Toxoplasma gondii*, the level of IL-5 was evaluated in different groups. Serological examination showed that the IgG antibody of Toxoplasmosis increased (P<0.05) in people with PD (65.71%). Serum levels of IL-5 significantly decreased in people with PD. It is noteworthy that comparing serum levels of IL-5 between two groups of people with and without chronic Toxoplasmosis revealed that there was a significant decrease (P<0.05) in a concentration of serum IL-5 in individuals with chronic Toxoplasmosis. The current study confirmed the conceivable association between *T. gondii* and PD, and further research is recommended to explain the association between PD and Toxoplasmosis.

Keywords: *Toxoplasma gondii*, Interleukin-5, Parkinson's disease

1. Introduction

Toxoplasma gondii (Apicomplaxa phylum), as an intracellular protozoan parasite with a complex lifecycle, is an opportunistic pathogen that infects a broad host range, sexual cycle in its feline definitive host, and asexual cycle in all warm-blooded animals, including humans. The conduct control theory recommends that parasites can modify in such a way that helps the parasite rather than the host (1, 2). The proliferation of the parasite in the host stimulates the immune system on the protozoan, leading to the formation of cysts that contain a slow-growing form (bradyzoite) in the nerve tissue and skeletal muscle and cause infection (3).

This parasite is a significant illustration of parasites that control the behavior of their hosts, and it has been found that rodents infected with this parasite are less sensitive to felines as the definitive host, which is important in the spread and epidemiology of the disease (4, 5).

Infection with *T. gondii* is often asymptomatic; however, in immunocompromised individuals, cysts containing bradyzoites can rupture, leading to the reactivation of the latent infection (3). Immune responses to *T. gondii* infection vary depending on the clinical signs of the disease. Local and systemic immune responses are dependent on history, host immune status, and genetic factors.

The results of epidemiological studies have shown that infection with Toxoplasmosis in some cases leads to mental and neurological diseases in individuals (5). Persistent infection with this parasite is associated with several diseases, such as Alzheimer's, schizophrenia, and Parkinson's (6). Moreover, discouragement, anxiety, fear or panic (7), epilepsy, bipolar confusion, and numerous instances of behavior problems have been recorded in infected people, such as suicide (8), traffic accident, crime and violence (9), and masochism (10).

Approximately 1% of the world's population is affected by Parkinson's disease (PD), and the main cause of PD remains unknown. Based on the findings of pieces of research, *T. gondii* affected the secretion of dopamine and other neurotransmitters in rodents and humans infected with the parasite. Dopamine imbalances in the brains of patients can be associated with mental disorders in patient (6). The results of studies have shown that in patients infected with *T. gondii*, the production of inflammatory mediators, such as cytokines, is altered. In neurological diseases, activated immune cells, such as monocytes or macrophages and T cells, secrete cytokines into the brain that affect the levels of cytokines in the blood (11, 12).

Therefore, the present study aimed to investigate the possible association between the *T. gondii* infection and levels of interleukin-5 (IL-5) that might have a certain impact on some pathological changes in Parkinson's patients.

2. Materials and Methods

2.1. Study Design Outline and Samples Collection

According to figure 1, blood samples were collected and divided into different groups, including penitents with Toxoplasmosis and Parkinson, penitents with Toxoplasmosis, penitents with Parkinson, and the control group. Samples of people with PD were collected from several provinces (e.g., Najaf, Babylon, and Baghdad, Iraq) and the control group was selected from Najaf Province, Iraq. The subjects were at the age range of 22-86 years, with a mean age of 53.63 years. The patients with PD aged 22-86 years, with a mean of 55.53 years, while the cases in the healthy group were at the age range of 28-85 years, with a mean of 52.28 years. A demographic form was designed to collect the information for all the participants (the appendix). The form included several questions, including name, gender, age, marital status, morbidity (other diseases, if any), pregnancies (for females), family history of PD, occupation, diagnosis of the stage of the disease, disease-specific treatment, duration of illness, and smoking.

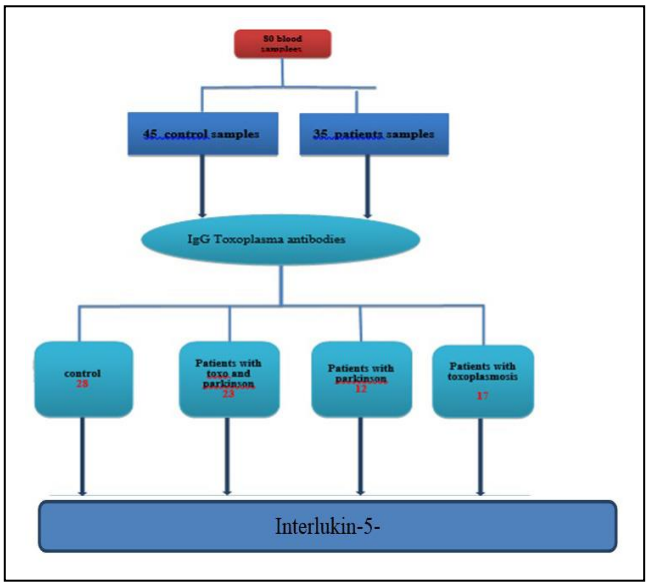

Figure 1. Study design outline

2.2. Immunoglobulin G and Interleukin-5 Measurements

An amount of 3 ml of venous blood was drawn aseptically from both patients and controls. The sera were separated through centrifugation at 3,000 rpm for 5 min, divided into four aliquots, and stored at -20°C until further use. The immunoglobulin G (IgG) antibody type to Toxoplasma parasite indicating chronic or latent infection was detected using the enzyme-linked immunosorbent assay (ELISA; Toxoplasma IgG ELISA kit, Calbiotech, California, USA). This ELISA facility pack utilizes the Sandwich-ELISA standard, and the serum levels of IL-5 were

measured using the IL-5 ELISA kit (Elabscience, Houston, Texas, USA) from January 2020 to April 2021 (cut-off value=calibrator mean OD×calibrator factor [0.5]) (Figure 2).

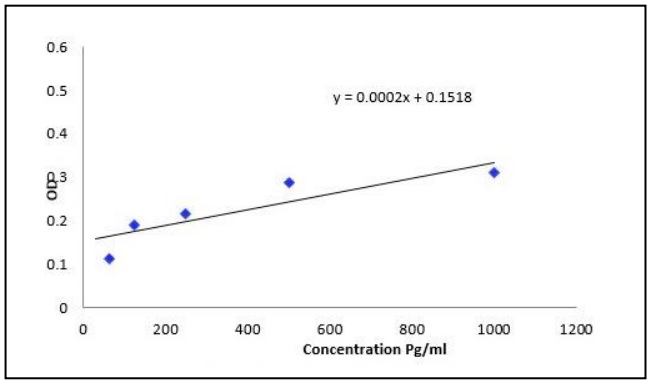

Figure 2. Standard curve obtained from the current study of interleukin-5

2.3. Statistical Analysis

Statistical evaluations were performed using the SPSS software (version 20; Armonk, NY, USA: IBM Corp.). For metric variables, mean±SD (minimum-maximum) was used, and for categorical variables, the percentage was employed as descriptive statistics. The analysis of variance test was utilized to compare the mean ages of groups. The χ^2 test was used to determine whether there was a difference between groups in terms of *T. gondii* seropositivity. A p-value of less than 0.05 was considered statistically significant.

3. Results

The patients' history showed that 35 subjects suffered from PD who were divided into two categories by specialists based on the severity of the disease, the essential stage (less severe; n=20), and the optional stage (more extreme; n=15). Furthermore, the medication history of infected cases indicated that 21 of them were receiving treatment (carbidopa-levodopa) and 14 patients did not receive medical care. (Table 1).

Serological examination of the samples followed by statistical analysis of the data showed that the chronic form of Toxoplasmosis (presence of IgG antibodies of *T. gondii* in the serum) increased (P<0.05) in people with PD (65.71%) (Table 2).

Table 1. Population or societal characteristics of the people under study

Treatment Carbidopa-Levodopa		Disease stages		female	male	Age Average age in years ± standard deviation (lower-higher)	Number	Group
Without treatment	therapy	secondary	primary					
14	21	15	20	16	19	55.53 ±17.29(22-86)	35	Patients with parkinson
0	0	0	0	12	33	(85-28)52.28 ±16.51	45	control
0	0	0	0	28	52	53.63±16.80(22-86)	80	total

Table 2. Percentage of IgG antibodies to Toxoplasma parasite in healthy people and people with Parkinson's disease

Propability	Ab negative	Ab positive	Total number	group
P value using chi square x2 test	28 (62.22%)	17 (37.77%)	45	Healthy (control)
0.01317=P	12 (34.28%)	23 (65.71%)	35	Patient with Parkinson
P<0.05	40 (50.0%)	40 (50.0%)	80	total

Serum levels of IL-5 were estimated using enzyme linked immunosorbent assay (ELISA) in the studied samples. The results of the t-test (P<0.05) showed that a significant contrast in IL-5 level led to a statistically significant decrease in individuals with Parkinson's disease (Table 3). It is noteworthy that when comparing serum levels of IL-5 between two groups of people with and without chronic toxoplasmosis, regardless of whether they had PD, it was found that there was a significant decrease (P<0.05) in the concentration of serum IL-5 among people with chronic Toxoplasmosis (Table 4). The use of *T. gondii* antibodies IgG in the serum of people under review led to the division of cases into different groups, including penitent with Toxoplasmosis and Parkinson, penitent with Toxoplasmosis, penitent with Parkinson, and control group. The serum levels of the IL-5 under study were compared between each of these groups (tables 4, 5).

Table 3. Serum levels of interleukin-5 in healthy people and people with Parkinson's disease

***P*. value**	**Concentration rate ± standard deviation M ± SD**		**Standards**
	Healthy subjects (control group) 45	**Parkinson's disease Patients 35**	
0.00044*	624.713 ± 544. 331	248.776 ± 302.092	Interleukin-5 Pg/ml

* Significant differences below the significance level of 0.05

Table 4. Serum levels of interleukin-5 in patients with Toxoplasmosis only

***P*. value**	**Concentration rate ± standard deviation M ± SD**		**Standards**
	Healthy 28	**Only people with toxoplasmosis 17**	
0.0775	705.669 ± 615.848	491.043 ± 379.854	Interleukin-5 pg/ml

* Significant differences below the 0.05 significance level

Table 5. Serum levels of interleukin-5 in Parkinson's patients only

***P*. value**	**Concentration rate ± standard deviation M ± SD**		**Standards**
	Healthy 28	**Parkinson's patients 12**	
0.0194*	705.669 ± 615.848	341.631 ± 409.942	Interleukin-5 Pg/ml

* Significant differences below the 0.05 significance level

Table 6 presents the effect of gender factors on the mean concentration of IL-5 in healthy and PD people. Statistical analysis using t-test showed that there was no significant difference (P>0.05) between men and women penitents in this regard. Based on the results of the t-test, there were significant differences (P<0.05) in interleukin-5; regarding this, a significant decrease was observed in people with PD, compared to the healthy group (Table 5). The findings showed that the stages and severity of PD had no effect on the concentration of IL-5. Moreover, there was no significant difference between people with different symptoms and degrees

of the disease. The effect of the treatment on the levels of IL-5 among people with PD was investigated. The statistical analysis with the t-test showed that IL-5 had no significant differences between the two groups.

Table 6. Serum levels of interleukin-5 in healthy individuals by sex

***P*. value**	**Concentration rate ± standard deviation M ± SD**		**Standard**
	Female 12	**Males 33**	
0.3381	572.587 ± 467.998	643.667 ± 575.090	Interleukin-5 Pg/ml

There are no significant differences below the significance level of 0.05

4. Discussion

The importance of Toxoplasmosis is due to the fact that not only it is a parasitic disease but also the life cycle of the parasite and the production of bradyzoite cysts in various tissues and organs of the body, especially the nervous system causes numerous complications in the host body. The results of studies have indicated that mental and neurological diseases caused by Toxoplasmosis infection are common (13). Additionally, the mice infected with Toxoplasmosis are not afraid of predators as definitive host, rather, they are attracted to them. Changes in behavioral patterns and neurological diseases have been observed in humans infected with *T. gondii* due to the proliferation of cysts in the nervous system (14).

In this study, a critical relationship was observed between Parkinson's disease as an important nervous disease and persistent Toxoplasmosis disease ($P<0.05$); regarding, *T. gondii* IgG antibody levels were significantly higher in Parkinson's patients (65.71%). This study was the first review to investigate the association between Toxoplasmosis and PD in Iraq.

In Iran, the findings of a study conducted by Mahami Oskouei, Hamidi (15) showed that there was no significant relationship between Parkinson's disease and Toxoplasmosis. However, a statistically significant association was found between PD and keeping cats and using undercooked eggs. Ramezani, Shojaii (16) found a significant relationship between Toxoplasmosis and PD, where IgG immune response rates were high in people with PD. Accordingly, the anti-Toxoplasmosis levels of IgG antibodies were significantly higher in the PD patients (82.5%), in comparison with healthy participants (65%) and patients with neurological disease (65.2%).

The relationship between PD and *T. gondii* infection by zero-molecular assessment showed that *T. gondii* infection could not only be a risk factor to PD but also put patients with PD at a higher risk of infection (6). The positive association between Toxoplasmosis and PD may be due to a number of factors, one of which may be a parasitic proliferation in the basal ganglia area for the cerebrum, which is the very significant physical site in the system of arrangement of PD (17). The results of this study indicated that the level of IL-5 was significantly higher in the two groups of cases with PD and Toxoplasmosis ($P<0.05$) than in the other groups.

Clarifying the role of lymphocytes in PD might lead to an identification of a common signature of lymphocytes in neuron degeneration and thus pave the road towards novel treatment options Physical and psychological stress stimulates the immune system, leading to the accumulation of inflammatory cells and the secretion of cytokines, leading to changes in synaptic levels and conduction changes. Parkinson's patients have clear evidence of accumulation of inflammatory cells. Examples of T cell invasion of the focal sensory system have been recorded in laboratory models (18).

When nerve damage and disease occur, the peripheral blood invades the brain with mononuclear cells or macrophages and T cells, which increases the secretion of cytokines (19). The results of a study have shown that in PD, due to the accumulation of immune cells in the brain, the level of cytokines changes (20). In the cerebrum, cytokines are created basically by microglia, however, are shared by astrocytes (21), neurons (22), and dendritic cells. In their creation, actuated glial cells seem valuable in the beginning phases of PD; nevertheless, when the infection advances, these cells have a reverse impact as they apply a neurotoxic impact (23). At the point when the cerebrum is damaged, cytokines are increased in the focal sensory system (24); therefore, glial cells begin to secrete neurotransmitters that affect nerve synapses and neuronal interactions. Stimulated glial cells produce chemokines, such as indolamine-2, 3-dioxygenase, inducible NO synthase, oxygen and nitrogen receptor species, which inhibit insensitive cells of the brain (19).Sawada, Imamura (25)showed that the inflammation caused by glial cell activity was an important factor in improving PD. Bardou, Kaercher (26)reported that some inflammatory drugs and cytokines (IL-1a, IL-2, GM-CSF, IL-4, IL-5, IL-6, IL-12, IL-13), including IL-5 increased in the focal area of the brainstem in the laboratory animals. In the current study, serum levels of IL-5 were compared between two groups, namely people with Toxoplasmosis and non-infected individuals, and it was revealed that, whether they had PD or not, there was a significant difference (P<0.05). The results showed that there was a significant reduction (P<0.05) in IL-5 of serum of Toxoplasmosis penitent, compared to that of healthy people, since microglia cells were the main factors that prevented the growth of microorganisms (27).

Interleukin-5 plays a vital role in the development of eosinophilic leukocytes, which assume a significant part in parasitic diseases. On the lethality of the assaulting parasite, the creation of IL-5 is connected to disease caused by worms and parasites (28). Interleukin-5 production has been observed during the period of *T. gondii* infection (29). Zhang and Denkers (30) investigated IL-5 levels during *T. gondii* infection among C57BL/6 mice. The results of the mentioned study indicated that mice with IL-5 deficiency developed proliferation. Interleukin-5 plays an important role in disease control during infection. Nickdel, Roberts (29) reported that IL-5 increased during the main period of infection (Acute), while it decreases in the second stage (Chronic).

Based on the findings of a study carried out by Delibas, Turgay (31), in the acute form of *T. gondii*, immunoglobulin M boosted with an increase in IL-5 and a decrease in interferon gamma (INF-γ), which indicated the predominance of the T2h (T-) profile; nonetheless, during the chronic period of the disease, an increase in IgG and INF-γ in serum and a decrease in IL-5 levels were observed. The results of the present study showed that factors, such as patient gender and disease severity, did not affect the level of IL-5.

The current study confirmed the conceivable association between *T. gondii* and PD. Parasitic infection and PD also affected some immunological perspectives by reducing IL-5 levels in both diseases. Further research is recommended to explain the association between PD and *T. gondii* and its effect on the severity of PD.

Authors' Contribution

Study concept and design: S. H. K. A.
Acquisition of data: B. A. A.
Analysis and interpretation of data: S. H. K. A.
Drafting of the manuscript: G. H. N. A.
Critical revision of the manuscript for important intellectual content: S. H. K. A.
Statistical analysis: S. H. K. A.
Administrative, technical, and material support: S. H. K. A.

Ethics

All studies were performed in compliance with the rules of humane treatment of Imam Ja'afar Al-Sadiq University, Al-Najaf, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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