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Evaluation of the Level of Immune Checkpoint Programmed Death Protein 1 (PD-1) in Toxoplasmosis

Ruqayah A. Abdul Ameer[1] *MSc*, **Huda D. Al-Marsomy**[2] *PhD*

[1]Dept. of Medical Laboratory Techniques, Sader Al-Iraq University College, Baghdad, Iraq ,[2]Dept. of Microbiology - College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background *Toxoplasma gondii* (*T. gondii*) is known to manipulate host immune responses to establish a persistent infection. This manipulation involves modulation of immune regulatory pathways, including those mediated by checkpoint molecules like programmed cell death protein 1 (PD-1). The parasite employs various strategies to evade host immune surveillance and promote its survival within the host organism.

Objective This study aimed to determine of the levels of PD-1 during acute T. gondii infection by enzyme linked immunosorbent assay (ELISA).

Methods In this case control study, samples were collected from 350 women suspected Tox with an age range from (18-48) years. According to the presence of anti-*T. gondii* (IgM) in serum of women using ELISA technique, the women were divided into 2 groups, group 1 with positive IgM, and group 2 with negative IgM. Serum levels PD-1 were measured in both groups.

Results The results showed a significant higher level serological biomarker (PD-1) in *T. gondii* IgM patients than in controls (p value 0.0001). Also, there was a significant variation of PD-1 level according to clinical history (p value 0.004), highest in those with delayed pregnancy, and lowest in those with cervical lymphadenopathy.

Conclusion There is a significant higher level serological biomarker (PD-1) in T. gondii IgM patients than in controls. Also, there is a significant variation of PD-1 level according to clinical history

Keywords Toxoplasmosis, IgM, PD-1.

List of abbreviations: CD4 = Cluster of differentiation 4, CD8 = Cluster of differentiation 8, ELISA = Enzyme linked immunosorbent assay, IRB = Institutional review board, IgG = Immunoglobulin γ (gamma), IgM = Immunoglobulin μ (mu), PD-1 = Programmed cell death protein 1, Pdcd1 = Programmed cell death protein 1 gene, PD-L1 = Programmed death ligand 1, Spp. = Species, *T. gondii* = *Toxoplasma gondii*, Tox = Toxoplasmosis

Introduction

Toxoplasmosis (Tox) is a coccidian and cosmopolitan disease in humans and animals caused by the opportunistic parasite named *Toxoplasma gondii* (*T. gondii*), it is an obligate intracellular parasite caused various disease [(1)]. A positive result for IgM antibodies against *T. gondii* in serological testing suggests recent infection [(2)]. Programmed cell death protein 1 (PD-1) is immune checkpoint protein that plays a crucial role in regulating T cell responses [(3)].

T. gondii is known to manipulate host immune responses to establish a persistent infection [(4)]. This manipulation involves modulation of immune regulatory pathways, including those mediated by checkpoint molecules like PD-1 [(5)].

The parasite employs various strategies to evade host immune surveillance and promote its survival within the host organism [6].

T. gondii infection can upregulate PD-1 expression on T cells, leading to T cell exhaustion and impaired immune responses against the parasite [7]. This manipulation of PD-1 signaling contributes to the parasite's ability to evade host immune surveillance [8]. Moreover, the interplay between *T. gondii* infection and the host immune system, mediated in part by PD-1, influences the outcome of the infection [9].

By modulating immune checkpoint pathways, the parasite can evade immune clearance and establish chronic infection [5]. In T cells, binding of PD-1 to one of its ligands induces the dephosphorylating of signaling intermediates downstream of the T cell receptor (TCR), leading to functional inhibition, upon chronic antigen exposure, PD-1 is persistently upregulated on T cells [10].

This study aimed to determine of the levels of PD-1 during acute *T. gondii* infection by enzyme linked immunosorbent assay (ELISA).

Methods

Study design

In this case-control, serum samples were collected from 350 women suspected infection with the *T. gondii* were selected from the Hospitals (Al-Imamein Al-Kadhimein Medical City, Kamal Al-Samarrai Hospital, Medical City, Al-Shaheed Mohammed Baqir Al-Hakeem General Hospital, Baghdad, Iraq), with an age ranged from (18-48) years. The diagnosis was conducted under the supervision of a consultant gynecologist. This study was conducted over a duration of eight months, specifically from September 2023 to June 2024. Ethical approval for this study (Approval No. 20230904) was obtained from the Institutional Review Board (IRB) of the College of Medicine, Al-Nahrain University, Baghdad, Iraq.

Inclusion criteria

Women with cervical lymphadenopathy, abortion, delayed pregnancy and flu-like symptoms (fever, headache, joint pain, cough, malaise, anorexia, nausea and pain in the body) were subjected for blood collection.

Exclusion criteria

Patients with other diseases such as (diabetes mellitus, under chemotherapy, other immune diseases).

Samples

Five milliliters (5 ml) of blood were obtained through venous puncture by the technicians. The serum was separated from the blood, and divided into three portions; the first portion was prepared for ELISA assay to detect *T. gondii* IgM positive and the second portion for ELISA assay to detect level of concentration to PD-1 proteins.

According to ELISA test for *T. gondii* IgM test, only 50 women were positive and considered as group 1, group 2 (control) included 50 individuals who were apparently healthy and revealed negative ELISA result for *T. gondii* IgM antibody.

Statistical analysis

T-test were used to compare between test groups and control. Chi square test was used to compare between frequency. The obtained data were subjected to one-way analysis of variance (ANOVA) test to compare various groups with each other. Results were expressed as mean±standard deviation (SD) and other descriptive statistics. Least significant difference (LSD) test was used to calculate the significant differences between tested mean, the letters (A, B, and C) represented the levels of significant, highly significant start from the letter (A) and decreasing with the last one. Similar letters mean there are no significant differences between tested mean. $P < 0.05$ considered as statically significant. The statistical analysis was

carried out by Statistical package for the social sciences (SPSS) version 20.

Results

Serum concentration of PD-1 in the study groups

The study revealed that the mean±SD of serum concentration of PD-1 was significantly higher in group 1 compared with group 2, (1490.87±250.1 vs 1474.0±189.15) (p value 0.0001) as shown in table (1).

Table 1. Comparison serum concentration of PD-1 between Tox IgM patients and control

Statistics	PD-1/ pg./ml	
	Tox IgM patients	Control
N	50	50
Mean	1490.87	801.14
Std. Error of Mean	35.37	26.75
Median	1474.00	835.88
Std. Deviation	250.10	189.15
Minimum	888.26	439.12
Maximum	2011.65	1170.89
P value	0.0001	

PD-1 serum concentration in Tox patients according to clinical history

Table (2) showed that there was a significant difference in PD-1 serum concentration in Tox patients according to the clinical history (P value 0.004), the highest concentration was found in women with delayed pregnancy (1494.04±282.52 pg/ml), a lower concentration was found in patient with abortion (1477.87±206.86 pg/ml), while the women with low cervical lymphadenopathy were had the lowest PD-1 concentration (1464.93±262.02 pg/ml) .

Table 2. Comparison of serum PD-1 concentration in Tox patients according to clinical history

PD-1 concentration	Abortion	Cervical lymphadenopathy	Delayed pregnancy
Mean	B 1477.87	C 1464.93	A* 1494.04
Std. Error of Mean	44.10	79.00	70.63
Median	1458.49	1361.64	1541.00
Std. Deviation	206.86	262.02	282.52
Minimum	1078.65	1190.85	888.26
Maximum	1860.16	1972.93	1884.74
P value 0.004			

*LSD test was used to calculate the significant differences between tested mean, the letters (A, B, and C) represented the levels of significant, highly significant start from the letter (A) and decreasing with the last one. P <0.05 considered statically significant

Discussion

The upregulation of PD-1 creates a potent immunosuppressive environment, allowing *T. gondii* to avoid immune clearance effectively. There are very few studies on the *T. gondii* and its immune checkpoint.

The presence of *T. gondii* IgM antibodies is a key marker in diagnosing acute Tox. During an acute infection, the immune system produces IgM antibodies as an early response to the parasite [(11)].

In Iraq, Mikaeel (2020) [(12)] concluded that presence of IgM antibodies was used to identify women with recent *T. gondii* infections. Also, Abd Al Ameer (2022) [(13)] said the presence of *T. gondii* IgM antibodies indicates acute or recent infection. Both studies used the presence of *T. gondii* IgM antibodies to identify recent infections and explore potential health impacts; these studies confirm the importance of monitoring *T. gondii* infections and their potential health effects.

The present study found a significant higher concentration of PD-1 levels in patients with acute Tox compared to control.

Khan et al. and Sharpe and Freeman [(14-15)] had found that immune responses in *T. gondii* infections highlighted that PD-1 expression is an important regulatory mechanism during both acute and chronic phases of infection. It modulates the immune response to prevent excessive inflammation and tissue damage.

This study revealed a significant difference of PD-1 serum concentration in Tox patients with clinical history; the highest concentration was in patients with delayed pregnancy, then patient with abortion, and the low cervical lymphadenopathy.

The study result is in agreement with Oliveira-Scussel et al. (2022) [(16)] who mentioned that during *T. gondii* infection, the modulation of immune responses by PD-1 can play a role in reproductive outcomes, including infertility.

Although direct studies on PD-1 expression in delayed pregnancy due to *T. gondii* are not available, the role of PD-1 in immune regulation during infections that can lead to infertility has been explored. Increased PD-1 expression can contribute to immune tolerance mechanisms that might influence fertility outcomes [(17)].

Dysregulation of PD-1 can contribute to adverse pregnancy outcomes, including abortion. While specific percentages in the topic of *T. gondii*-induced abortion are not detailed, PD-1's role in immune regulation is significant in this topic [(18-19)].

PD-1 expression is upregulated to modulate the immune response and prevent excessive inflammation and tissue damage. This regulation is critical in lymphoid tissues, such as lymph nodes, where immune responses are initiated and regulated [(20)], also PD-1 is crucial for maintaining immune homeostasis during

infections. Upregulation of PD-1 during *T. gondii* infection helps in controlling the immune response, which can be reflected in lymphadenopathy where immune cells are actively responding to the infection [21].

In conclusion, there is a significant higher level serological biomarker (PD-1) in *T. gondii* IgM patients than in controls. Also, there is a significant variation of PD-1 level according to clinical history.

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Author contribution

Abdul Ameer: Sample collection, laboratory work, statistical analysis, and writing the paper manuscript. Al-Marsomy: Study design, supervision of the laboratory work and final revision of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Correspondence to Ruqayah A. Abdul Ameer
Email: ruqayah.msmm23@ced.nahrainuniv.edu.iq
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