Original Article

The effect of infection with *Toxoplasma gondii* in inducing interferon-gamma in breast cancer patients

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ABSTRACT

Toxoplasmosis is one of the most widespread zoonotic diseases in the world. Human infection rates range from 10% to 80% in many countries. Female cancer patients receiving chemotherapy are more susceptible to developing acute forms of toxoplasmosis, which can cause brain defects, neurological damage, and encephalitis. The aim of the present study was to investigate the effect of Toxoplasma gondii infection on the induction of interferon-gamma in breast cancer patients from Iraq. This descriptive cross-sectional study was performed on women had breast cancer in Al-Haboubi Teaching Hospital in Nasiriya City-Thi-Qar Province (Iraq) during the period from January to September 2022. Approximately three ml of blood was drawn from all participants and sera were collected. The Sera were then tested for *Toxoplasma* IgM, IgG, and IFN-γ (Nova Tec Immunodiagnostica GmbH, Germany) using the enzyme-linked immunosorbent assay (ELISA) kits according to the protocols of the manufacturer. Before blood collection, participants completed a printed questionnaire with some demographic information, such as age and place of residence. The total number of positive T. *gondii* infections from breast cancer patients in the current study was 60 (85.7%). The results of sample analysis by ELISA assay showed that 85.7% and 74.2% of patients were positive for IgG and IgM, respectively. The mean IFN-γ levels in breast cancer patients with toxoplasmosis, without toxoplasmosis, and in the control group were 47.66, 0.00, and 0.57 pg/ ml, respectively. Higher IgG and interferon gamma levels were detected in the group of breast cancer patients with toxoplasmosis than in the group without toxoplasmosis. According to the ELISA findings, *T. gondii* was the most common parasite in female cancer patients.

Keywords: Toxoplasmosis; cancer; Enzyme-linked immunosorbent assay; antibody; serology

1. Introduction

Toxoplasmosis is one of the most widespread zoonotic diseases in the world. Human infection rates range from 10% to 80% in many countries (1). *Toxoplasma gondii*, the most successful parasite in the world, can infect almost any nucleated cell in animals, including birds and mammals (1). *T. gondii* is usually transmitted in one of the following ways: by eating contaminated undercooked meat, by eating oocysts shed by cats, or by congenital transmission: when a mother is first infected with toxoplasmosis during pregnancy, it is likely that the fetus will also become infected (1,2). Although *T. gondii* infection rarely causes symptoms in immunocompetent people, it can have serious health consequences or even be fatal in immunocompromised people (2). Cancer patients receiving chemotherapy are more susceptible to developing acute forms of toxoplasmosis, which can cause brain defects, neurologic damage, and encephalitis (2). Detection of parasite antibodies is the most common method for diagnosing toxoplasmosis (specific IgG and IgM antibodies) in serum samples. Meanwhile, (ELISA) is the most commonly used serological method (1). *T. gondii* colonizes intracellularly and can migrate through the extracellular space to find new host cells, activating cellular and humoral immunity (3). Patients with toxoplasmosis exhibited varying serum levels (cluster of differentiation 4 (CD4), IFNγ, interleukin (IL)-4, IL12, and IL23) before, during, and after infection (3). The early processes leading to the management of *T. gondii* infection are the synthesis of IL-12 and the induction of natural killer (NK) and T cells to produce IFNγ. In this context, it is necessary to control chronic and acute infections. Innate lymphoid cells type I (ILC1), ILC2, and ILC3 are the three subsets of ILC. IFN-γ and TNF-α were produced by group one ILC1 in response to oral infection in the small intestine. NK cells, ILC1, and T cells are at least three different cell types that control IFN-γ production during infection. These cells can balance each other (4). Breast cancer is the second most common disease in women after skin cancer. Breast cancer is the growth of cancer cells in breast tissue, which can occur in either the mammary ducts or the mammary glands, with the type in the mammary ducts usually being the more common type. In Iran, more than 10,000 people are diagnosed with breast cancer every year, and this number increases by 5% every year (5). The aim of the present study was to investigate the effect of *T. gondii* infection on the induction of interferon-gamma in breast cancer patients from Iraq.

2. Materials and Methods

2.1. Participants

This descriptive cross-sectional study was conducted on women who had breast cancer in Al-Haboubi Teaching Hospital, Nasiriya City-Thi-Qar Province (Iraq) during the period from January to September 2022 (all patients were examined by a gynecologist).The present study also included 70 healthy individuals without current or previous cancer who were referred to hospitals during the above study period and were part of the control group. Participants who had taken systemic antibiotics in the last three months and immunocompromised patients were excluded.

2.2. Questionnaire

Before blood collection, participants completed a printed questionnaire with some demographic information, ,such as age and place of residence, was completed for participants.

2.3. Sample collection

Three millilitres of blood were drawn from all participants. Blood was collected under sterile conditions by venipuncture using 3- ml disposable plastic syringes. The blood was collected in a gel tube, where it was allowed to clot at room temperature for one h. After the blood had clotted, it was centrifuged at 4000 rpm for 10 min . The serum was then divided evenly into two portions and placed into Eppendorf tubes for use in immunological assays (*T. gondii* IgG, IgM, and IFN-γ). Finally, the sample was stored at 20°C. To avoid repeated freezing and thawing, only one of each sera component was used.

2.4. Evaluation of T. gondii Antibodies

All collected sera were tested with the (ELISA) kits for *Toxoplasma* IgM, IgG, and IFN-γ (Nova Tec Immunodiagnostica GmbH, Germany) according to the protocols of the manufacturer using an ELISA reader (BIOTEK ELX800TS, USA).

2.5. Statistical analysis

SPSS software (ver. 24) was used for data analysis. Chi-square and logistic regression tests were used to compare the association between variables. $P < 0.05$ indicated a significant difference.

3. Results

The results showed that 85.7% of cancer patients were positive for *T.gondii* infection, while 14.2% of them were negative for toxoplasmosis ($P<0.001$).Table 1 shows the frequency of *T. gondii* IgG and IgM antibodies in breast cancer patients and healthy individuals. The results showed a significant difference in the study of *T. gondii* IgM infection and patients with breast cancer. The rate of *T. gondii* IgM antibodies in patients with breast cancer

was reported to be 74.2%, while 25.7% of breast cancer patients were negative (Table 1).

Table 1. Frequency of *Toxoplasma gondii* IgG and IgM antibodies in breast cancer patients and healthy people.

Group	IgG antibody		P value	IgM antibody		P value
	Positive No. (%)	Negative No. (%)		Positive No. (%)	Negative No. (%)	
Brest Cancer patients	**60 (85.7)**	**10(14.2)**	**0.001***	**52 (74.2)**	**18 (25.7)**	**0.001***
Healthy people	**0 (0.0)**	**20 (100)**	-	**0 (0.0)**	**20 (100)**	-

* Significant difference compared with the healthy people

As shown in Table 2, the age of breast cancer patients infected with *T. gondii* ranged from 21-80, with a high rate of infection (35%) with *T. gondii* observed in the age group of 41-50 years. In contrast, the lowest infection rate (5%) with *T. gondii* was observed in cancer patients in the age group of 21-30 years. There was a significant association between the prevalence of *T. gondii* antibodies and the age group of 41-50 years (P< 0.001). The infection rate of *T. gondii* was 43.3% among cancer patients living in an urban area. In comparison, the infection , rate with *T. gondii* was 56.6% among cancer patients living in rural areas. Statistical analysis showed no significant difference between the residence of cancer patients and *T. gondii* infections.

Table 2. Comparison of *Toxoplasma gondii* in breast cancer patients based on the associated risk factors by regression analysis.

Group	Positive No. (%)	Crude OR	95%CI	P value
Age (yrs)				
10-20 yrs	0.00			
21-30 yrs	3 (5.0)			
31-40 yrs	5 (8.3)			
41-50 yrs	21 (35.0)	0.067	0.019-0.253	<0.001*
51-60 yrs	17 (28.3)			
60-70 yrs	9 (15.0)			
70-81 yrs	5 (8.3)			
Residence **Rural** **Urban**	 34 (56.6) 26 (43.3)	 - 0.291	 - 0.75-11.27	 - 0.123

* Significant difference compared with the healthy people

In the following study, IFN-γ levels were checked in breast cancer patients with or without *T. gondii* (IgG) The results showed a high IFN-γ level in breast cancer patients with *T. gondii* (47.66 pg/mL) compared with a decreasing IFN-γ level (0.00) in those not infected with *T. gondii* (IgG) (Table 3).

Table 3. The serum level of Interferon-gamma (IFN-γ) in the participants.

Group	Mean of IFN-γ (pg/mL)	P value
IgG (+) **Brest Cancer patients** **IgG (-)**	47.66 0.00	<0.00* -
IgG (+) **Healthy people** **IgG (-)**	0.00 0.57	- -

* Significant difference compared with the healthy people

4. Discussion

T. gondii is a widespread, obligatory intracellular parasite that is widely distributed worldwide.The most common type of infection, it is generally asymptomaticbut can lead to opportunistic infections in immunocompromised individuals (6-8). In addition, toxoplasmosis is believed to contribute to the development of cancer. Numerous studies have shown that seroprevalence rates of toxoplasmosis are much higher in people with cancer, particularly breast cancer,

than in people without cancer (9). Breast cancer is an abnormal proliferation of the excretoryducts and cells that line the breast branches. These cells can spread to other cells, tissues, and body organs because of their irregular and unpredictable proliferation (9). In 2012, approximately 1.7 million new breast cancer patients were reported . This makes breast cancer the second most common cancer after lung cancer (10). Risk factors that trigger breast cancer include a family history of the disease, obesity, nonbreastfeeding , alcohol consumption, and hormonal disorders (9). The aim of the present study is to determine the frequency of *T. gondii* infection in women with breast cancer, since this cancer is more common than other cancers in Thi-Qar Province (Iraq). The total number of positive patients for *T. gondii* infection in the current study was 60 (85.7%). The results of analysis of samples by ELISA test showed that 60/70 breast cancer patients were infected with *T. gondii*. Examination of serum samples from breast cancer patients for for anti-toxoplasmosis antibodies showed that 85.7% of them were positive for IgG, which was due to persistent latent infection acquired in the past, associated with high IgG levels (11). The prevalence rate of *T. gondii IgM* antibodies was found in 74.2% of breast cancer patients. This is often due to decreased host immunity in cancer patients, which may reactivate a previous chronic *T. gondii* infection or raise the possibility of reinfection (12). The present study was supported by other studies conducted in the Iraqi provinces of Basrah, Babylon, and Baghdad (13-15). However, our results do not agree with the results of studies conducted on cancer patients in Saudi Arabia, Egypt, and China (16, 17). According to the results of the current study, the 41-50 year-old age-group is most affected by *T. gondii* infection. The explanation is that immune function is weakened in cancer patients, which is the main cause of the increase in Toxoplasma infections in cancer patients (14). Our results are consistent with those of other studies conducted in Basrah Province of Iraq and Saudi Arabia (13, 15). The current study is in contradiction with the studies conducted in Baghdad province of Iraq and Egypt (16, 17). The distribution of *T. gondii* infections by place of residence was higher in rural areas (56.6%) than in urban areas (43.3%) in the current study.The results for cancer patients in the present study were consistent with a study conducted in Malaysia (18). Interferons are a group of proteins (cytokines) that are critical for mammalian host resistance to infection. Cells infected with viruses release type I interferons, including IFN- α and IFN- β. Under certain activation conditions, both T cells and natural killer (NK) cells release II type interferon and IFN-γ (19). The function of IFN-γ in enhancing host immunity against microbes is to regulate various components of the immune response , stimulating antigen presentation via class I and class II MHC, stimulating phagocyte activity, and affecting cell growth and apoptosis. This infection is critical for immune surveillance in cancer, supports antitumor immunity, and contributes to tumor identification and removal (19). *T. gondii*, as a parasitic infection with protozoa, can enhance resistance to certain types of tumors. Although very severe *T. gondii* infection is associated with excessive IFN-γ production, this immune activation causes the parasite to transform into bradyzoites, prevents parasite development, and limits tissue spread (20). IFN-γ is essential for *T. gondii* infections, both during chronic infection to maintain latent infection and prevent reactivation of infection, and during the acute phase of infection to limit the proliferation of tachyzoites (19, 20). According to the current study, the mean IFN-γ level in breast cancer patients with toxoplasmosis was 47.66 pg/ ml, whereas it was 0.00 in breast cancer patients without toxoplasmosis.This difference may be due to the fact that *T. gondii* infection increases interferon-gamma levels more than in healthy individuals through early T-cell and NK-cell activation (21). Cancer patients also have lower numbers of B and T cells (CD4 and CD8) in their blood, which could significantly affect immunological function (22).This study is consistent with previous studies measuring INF-gamma and IL17 in breast cancer patients infected with and without *T. gondii* treated in the oncology department of Al-Diwanyiah and Baghdad provinces in Iraq (23, 24) and in Egypt and Brazil (17, 25). In conlcusion, although the small sample size was the major limitation of the present study, higher IgG and interferon-gamma levels were detected in the breast cancer patients with toxoplasmosis compared with the group without toxoplasmosis.According to the ELISA findings, *T. gondii* was the most common parasite in female cancer patients.

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Authors' Contribution

AKK and FAH contributed to the study concept and design. AKK and FAH collected the data. AKK drafted the manuscript. All authors read and approved the final manuscript.

Ethics

Patient enrollment was performed in line with the 1964 Helsinki declaration. The study was reviewed and received the ethical approval from the ethics committee of University of Thi-qar, Thi-qar, Iraq (No. 2022234). However, a printed informed consent was attained from all participant before registration. The authors confirm that all methods were carried out in accordance with relevant guidelines and regulations.

Conflict of Interest

The authors declare that they have no competing interests.

Availability of data and materials

All data is available in the text of the manuscript and Tables.

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