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Original Article

Frequency of Anti-*Toxoplasma gondii* IgM and IgG and the Associated Risk Factors in Patients with Breast Cancer: A Case-Control Study

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Abstract

Background: *Toxoplasma gondii* is an obligate intracellular parasite capable of causing serious disease, especially among individuals with compromised immunity, including those undergoing cancer treatment. Approximately one-third of the global population is infected with. *gondii*. The present investigation evaluated the prevalence of *anti-T. gondii* IgM and IgG antibodies and explored related risk factors in breast cancer patients compared with healthy controls.

Methods: Between Oct 2023 and Feb 2024, serum samples were obtained from 167 women, including 111 with breast cancer and 56 healthy controls. *Anti-T. gondii* IgM and IgG antibodies were measured through ELISA. Structured questionnaires were employed to record demographic profiles and relevant exposure factors. Data were statistically processed using chi-square, t-test, and logistic regression modeling.

Results: Overall, the IgG antibody was positive in 48.6% of the cases with breast cancer and 33.9% of the control group. However, the groups had similar mean *T. gondii* IgG antibody titers, with no significant differences. Moreover, the IgM antibody tests were negative in both groups. Our results show a high seropositivity rate for toxoplasmosis in age groups over 50 yr old. No meaningful correlations were identified between seropositivity and other factors such as dietary habits, contact with animals, or history of blood transfusion.

Conclusion: Anti-*T. gondii* IgG seropositivity was higher among breast cancer patients than controls, but the association was not statistically significant after adjustment for potential confounders. These findings do not provide sufficient evidence of an association between *T. gondii* seropositivity and breast cancer. Further studies with larger sample sizes are warranted to clarify this relationship.

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Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan that infects numerous species, humans among them. This parasite causes toxoplasmosis, and global estimates suggest that nearly one in three individuals is infected (1, 2). The global prevalence of *T. gondii* varies considerably based on geographic location, socioeconomic status, and dietary habits. The primary routes of infection include ingesting food or water contaminated with oocysts, consuming undercooked meat containing tissue cysts, blood transfusion, congenital transmission, and organ transplantation (2).

Routine diagnostic methods for toxoplasmosis include enzyme-linked immunosorbent assay (ELISA) (3), immunofluorescence antibody assay, and chemiluminescent immunoassay (CLIA) (4). In these serological methods, infection with the *T. gondii* parasite is usually diagnosed by detecting parasite-specific antibodies (i.e., IgM and IgG) in patient serum samples (5, 6).

Although this parasite causes inflammation in healthy people, (7) infection with this parasite is usually asymptomatic. Still, patients with suppressed immune function, such as patients with acquired immunodeficiency syndrome (AIDS), cancer, and transplant recipients, display reduced immune effectiveness in combating the parasite. This causes acute infection by transforming the resting or bradyzoite stage into the tachyzoite, or active form (8). *T. gondii* can cause life-threatening infections that lead to severe health complications in immunocompromised individuals, such as cancer patients (9). Neurological symptoms of toxoplasmosis in immunocompromised individuals include hemiparesis, headache, drowsiness, numbness, seizures, and reflex changes (10, 11).

One of the primary causes of death is cancer, and according to the available estimations, this disease stands as the second major cause of death after heart disease (12). Cancer patients, especially those undergoing chemotherapy, are at high risk for developing toxoplasmosis due to their weakened immune systems, and multiple types of malignancies, such as lymphoma, myeloma, and leukemia, can cause reactivation of toxoplasmosis. Breast cancer ranks as the most prevalent cancer in women and, after lung cancer, is the second major cause of cancer-associated deaths, causing significant morbidity and mortality at all ages worldwide (13). Therefore, the factors that cause breast cancer or increase its mortality are substantial (14).

The seroprevalence of *T. gondii* in different population groups is of great importance as it helps to understand the extent of exposure in those groups and the potential need for preventive measures (1). Most previous studies have shown that individuals with cancer are more frequently infected with *T. gondii* than healthy populations (15).

Given the high prevalence and potential severity of *T. gondii* infection among individuals with cancer, preventive strategies and appropriate monitoring of this vulnerable population are important (15). In addition, because of the importance of breast cancer and its high incidence and mortality, collecting reliable clinical information on toxoplasmosis prevalence in this patient group is essential (14). Thus, this study aimed to compare the anti-*T. gondii* IgM and IgG seroprevalence and determine associated risk factors in patients with breast cancer.

Given the high prevalence and potential clinical consequences of *T. gondii* infection in immunocompromised individuals, determining its seroprevalence among patients with cancer is of considerable importance (15). Although previous studies have reported increased *T. gondii* seropositivity in cancer patients, evidence specifically concerning women with breast cancer remains limited and incon-

clusive (16, 17). Moreover, substantial geographic, environmental, and exposure-related variations in *T. gondii* seroprevalence limit the generalizability of previous results, and it remains unclear whether breast cancer is independently associated with seropositivity after adjustment for potential confounding factors.

Materials and Methods

This case-control study was conducted with the approval of the Ethics Committee of Gonabad University of Medical Sciences (IR.GMU.REC.1403.077).

The present study included 167 women who were referred to Fayyaz Bakhsh Hospital in Tehran between Oct 2023 and Feb 2024 and were subsequently enrolled in the study. Of these, 111 women were diagnosed with breast cancer, and 56 women from the hospital's outpatient clinics served as the control group, with an age range of 25-65 yr. Participants were recruited using convenience sampling. The control group was screened using a structured questionnaire and face-to-face interviews to exclude participants with malignancy, immunodeficiency, and other exclusion criteria. Individual matching was not performed; however, both groups were selected from the same hospital, during the same study period, and within the same age range. Participants with known immunodeficiency, HIV infection, organ transplantation, or malignancy were excluded. Detailed data on cancer stage and treatment status were not available during the study period.

Before sampling, all participants provided verbal informed consent. Written informed consent was not obtained because the Ethics Committee approved the use of verbal informed consent for this minimal-risk observational study.

The questionnaire covered questions related to age, marital status, education, and potential risk factors associated with the prevalence of toxoplasmosis, such as eating habits, history of blood transfusion, ownership of cats or dogs, direct contact or handling of domestic animals, contact with soil, and consumption of raw or undercooked meat and improperly washed vegetables.

Venous blood samples (about 5 mL) were taken from each participant into sterile gel clot activator vacuum tubes and allowed to rest at room temperature for 30 min to form clots. Afterward, the samples underwent centrifugation at 1,640 × g for 8 min, after which the supernatants were transferred to Eppendorf tubes and stored at -20 °C until further examination. The presence of anti-*T. gondii* IgM and IgG antibodies was assessed via ELISA using a commercial kit (Pishtaz Teb, Tehran, Iran) following the manufacturer's instructions. IgM and IgG antibody levels were interpreted according to cut-off values, with readings above 11 IU/mL considered positive and those below 11 IU/mL considered negative.

Sample Size Calculation

Based on previous studies (18) that investigated the potential role of *T. gondii* infection in breast cancer and found that the seropositivity for anti-*T. gondii* IgG antibodies was 47.30% in the case group and 16.60% in the control group. Then, using the following formula and considering a type I error of 0.05 ($\alpha = 0.05$), power of 90%, $p_1 = 0.16$ (control group), and $p_2 = 0.47$ (case group), the minimum required sample size in each group was obtained as 41 samples. Finally, by considering 30% attrition and to increase the power of the study as well, 167 patients were considered in the study in total (allocation ratio (N2/ N1): 2:1).

$$n = \frac{(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 * [p_1 * (1-p_1) + p_2 * (1-p_2)]}{(p_1 - p_2)^2} = \frac{(1.96+1.24)^2 * (0.16*0.84+0.47*0.53)}{(0.16-0.47)^2} \cong 41$$

Power Analysis

We performed a power analysis using the power two proportions command in Stata (ver. 14.1). The calculated sample size provides > 90% power to detect the specified difference.

Statistical analysis

We reported means ± standard deviations and frequencies (%) to summarize quantitative and qualitative variables. To investigate associations between qualitative variables, the Chi-square and Fisher's exact tests were used. We compared mean values of quantitative variables between the two study groups using an independent t-test. Moreover, a multiple logistic regression model was fitted to explore the influence of multiple predictors simultaneously (19). The model was applied to investigate the factors associated with having anti-*T. gondii* IgG seropositivity, followed by adjustment for potential confounders. Thus, a univariate logistic regression model was first fitted to the data for variable selection. Subsequently, all variables with a $P<0.25$ were included in a multiple logistic regression model. All statistical analyses were conducted using SPSS ver. 20.0 (IBM Corp., Armonk, NY, USA), with a significance level set at 0.05.

Results

Out of 167 patients in the study, 56 (33.5%) were in the control group (mean age 45.41 ± 15.67 yr), and 111 (66.5%) were in the case group (mean age 50.00 ± 10.12 yr). The independent t-test showed no statistically significant difference in mean age between the two groups ($P>0.05$). Table 1 presents the baseline characteristics of patients in both groups. Results from the Chi-square test showed that the frequency distribution of education level and smoking between the two case and control groups had statistically significant differences ($P<0.05$). No other variables showed a statistically significant difference between groups ($P>0.05$).

Table 1: Baseline characteristics of the study groups

Variables		Groups		*P*- value
		Control	Case	
Age (yr)	Mean±SD	45.41±15.67	50.00±10.12	0.17
	≤ 50	34 (60.70)	61 (55.00)	0.47
	> 50	22 (39.30)	50 (45.00)	
Education	Under diploma	40 (71.40)	50 (45.00)	0.001*
	Diploma and higher	16 (28.60)	61 (55.00)	
Marital status	Single	7 (12.50)	8 (7.20)	0.25
	Married	49 (87.50)	103 (92.80)	
Obesity history	No	47 (83.90)	99 (89.20)	0.33
	Yes	9 (16.10)	12 (10.80)	
Job	Housewife	2 (3.60)	9 (8.10)	0.25
	Employee/retired	6 (10.70)	19 (17.10)	
	Freelance	23 (41.10)	49 (44.10)	
	Other	25 (44.60)	34 (30.60)	
Smoking	No	47 (83.90)	104 (93.70)	0.04*
	Yes	9 (16.10)	7 (6.30)	
History of alcohol use	No	54 (96.40)	111 (100.00)	0.11
	Yes	2 (3.60)	0 (0.00)	

Values are given as frequency (%) or mean ± SD; * Significant at the level of 0.05

Table 2 assesses the association between anti-*T. gondii* IgG and baseline characteristics and dietary habits of patients in the two groups. Considering the control group, only the age

group showed a statistically significant association with anti-*T. gondii* IgG seropositivity ($P<0.05$), while no such association was observed for the other variables in both groups ($P>0.05$).

Table 2: Baseline characteristics and dietary habits of patients associated with anti-*T. gondii* IgG seropositivity

Variables		IgG results in the control group		*P*	IgG results in the case group		*P*
		Negative	Positive		Negative	Positive	
Age(yr)	Mean±SD	44.89±16.11	46.42±15.16	0.73	48.56±9.32	51.52±10.79	0.12
	≤ 50	26 (70.30)	8 (42.10)	0.04*	36 (63.20)	25 (46.30)	0.07
	> 50	11 (29.70)	11 (57.90)		21 (36.80)	29 (53.70)	
Education	Under diploma	25 (67.60)	15 (78.90)	0.37	25 (43.90)	25 (46.30)	0.79
	Diploma and higher	12 (32.40)	4 (21.10)		32 (56.10)	29 (53.70)	
Marital status	Single	6 (16.20)	1 (5.30)	0.24	4 (7.00)	4 (7.40)	0.93
	Married	31 (83.80)	18 (94.70)		53 (93.00)	50 (92.60)	
Water type	Tap	37 (100.00)	19 (100.00)	-	44 (77.20)	48 (88.90)	0.10
	Bottled/ boiled/ Purified	0 (0.00)	0 (0.00)		13 (22.80)	6 (11.10)	
Job	Housewife	1 (2.70)	1 (5.30)	0.99	3 (5.30)	6 (11.10)	0.14
	Employee/retired	4 (10.80)	2 (10.50)		13 (22.80)	6 (11.10)	
	Freelance	15 (40.50)	8 (42.10)		21 (36.80)	28 (51.90)	
	Other	17 (45.90)	8 (42.10)		20 (35.10)	14 (25.90)	
Obesity history	Yes	5 (13.50)	4 (21.10)	0.46	9 (15.80)	3 (5.60)	0.08
Smoking	Yes	6 (16.20)	3 (15.80)	0.99	5 (8.80)	2 (3.70)	0.43
History of alcohol use	Yes	1 (2.70)	1 (5.30)	0.99	0 (0.00)	0 (0.00)	-
Contact with dogs and cats	Yes	0 (0.00)	1 (5.30)	0.33	4 (7.00)	2 (3.70)	0.67
Vegetable consumption	Yes	26 (70.30)	13 (68.40)	0.88	46 (80.70)	45 (83.30)	0.71
sausages or hot dogs consumption	Yes	24 (64.90)	13 (68.40)	0.79	22 (38.60)	27 (50.00)	0.22
Contact with soil	Yes	11 (29.70)	6 (31.60)	0.88	9 (15.80)	9 (16.70)	0.90
History of receiving blood	Yes	2 (5.40)	2 (10.50)	0.48	13 (22.80)	14 (25.90)	0.70

Values are presented as frequency (percentage); * Significant at the level of 0.05

Fig. 1 depicts the frequency of anti-*T. gondii* IgM and IgG results in both groups. No participants in either group were positive for anti-*T. gondii* IgM. Positive anti-*T. gondii* IgG results were more common in the case group than in the control group (48.6% vs. 33.9%), yet the difference was not statistically significant according to Chi-square analysis ($P=0.07$).

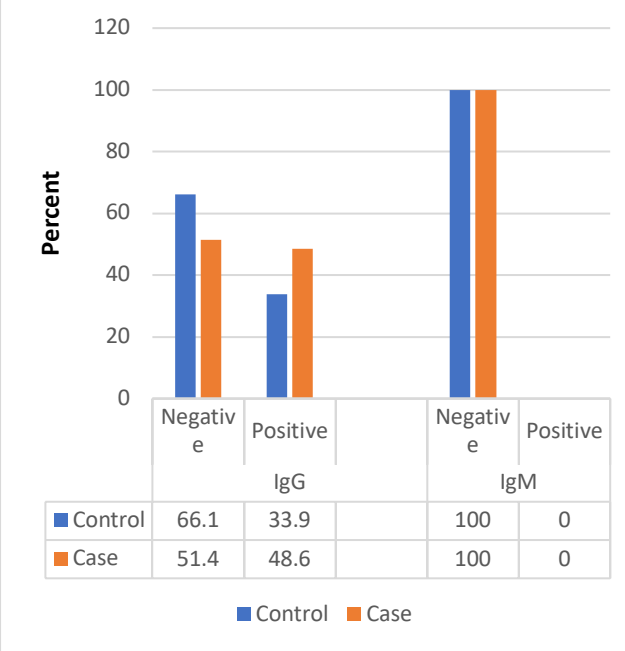


Fig. 1: Frequency distribution of anti-*T. gondii* IgM and IgG test results among case and control groups

Results from the multiple logistic regression model, after adjusting for the effects of smoking, education level, obesity history, sausage or hot dog consumption, water type, and history of receiving blood transfusions, indicated no statistically significant difference in the odds of a positive anti-*T. gondii* IgG test between the control and case groups. With adjustment for other variables, the odds of a positive *anti-T. gondii IgG* test were 1.85 times higher in the case group than in the control group, but the difference did not reach statistical significance (P>0.05). Upon controlling for the effect of other variables in the model, age was significantly positively associated with the odds of a positive anti-*T. gondii* IgG test, which was 2.80 times higher in individuals aged over 50 yr compared to those under 50 yr (P<0.05). However, the other variables were not significantly associated with the odds of testing positive for anti-*T. gondii* IgG (P>0.05) (Table 3). The goodness-of-fit of the final multiple logistic regression model was evaluated using the Hosmer-Lemeshow test. The final model has an acceptable fit to the data (Hosmer-Lemeshow χ^2(df=47)= 47.35, P=0.45).

Table 3: Determining the association between anti-*T. gondii* IgG seropositivity and breast cancer by adjusting for the effect of potential confounders using the multiple logistic regression model

Variables (References)	OR## (%95 CI)	*P*-value
Group (Control)	-	-
case	1.85 (0.86, 4.00)	0.11
Age (≤ 50 y)	-	-
> 50 y	2.80 (1.29, 6.07)	0.009*
Smoking (20)	-	-
Yes	1.50 (0.44, 5.02)	0.50
Education (Under diploma)	-	-
Diploma and higher	1.24 (0.57, 2.70)	0.57
Obesity history (20)	-	-
Yes	0.54 (0.19, 1.53)	0.24
Sausages or hot dog consumption (20)	-	-
Yes	1.40 (0.71, 2.75)	0.32
Water type (Tap)	-	-
Boil/Mineral/Purified	0.55 (0.18, 1.66)	0.29
History of receiving blood (20)	-	-
Yes	1.09 (0.46, 2.58)	0.83

* Significant at the level of 0.05; # OR=Odds Ratio

Discussion

The study examined the seroprevalence of IgM and IgG antibodies against *T. gondii* among women with breast cancer compared with healthy individuals, and identified factors that may contribute to infection. The prevalence of anti-*T. gondii* IgG antibodies was higher among breast cancer patients (48.6%) than in the control group (33.9%), but the difference was not statistically significant. Our results are supported by previous studies showing a higher rate of chronic *T. gondii* infection in immunocompromised populations, including those with cancer (21).

Moreover, the IgM antibody tests were negative in both groups, suggesting no active or recent infections, consistent with findings in similar studies involving cancer patients (22). For example, in a previous study, the seroprevalence of IgG antibodies against *Toxoplasma gondii* was reported to be 45.8% in patients diagnosed with breast cancer in Jahrom, located in southern Iran. Notably, all samples tested negative for anti-*T. gondii* IgM antibodies (16). The similar IgG seroprevalence observed in our study and in the Jahrom study may reflect comparable exposure in these Iranian populations, although regional differences and variation in patient characteristics should be considered. Differences in serological assays and study populations may also contribute to the variability in reported estimates.

However, this parasite can reduce cancer cell proliferation and metastatic behavior, and it has been suggested to inhibit cell proliferation in cancer development (23).

Previous studies have reported inconsistent associations between *T. gondii* infection and cancer, potentially reflecting differences in cancer type, diagnostic methods, and patient characteristics. Findings from hematological malignancies or brain tumors may not be directly generalizable to breast cancer (23, 24). Moreover, serological and molecular studies assess different aspects of infection. Cancer stage, treatment, and immune status may also influence the findings; however, these factors were unavailable in our study, limiting further comparisons.

The results of our study showed that age was associated with anti-*T. gondii* IgG seropositivity. This association remained evident in the multivariable analysis, in which participants older than 50 yr had 2.80-fold higher odds of anti-*T. gondii* IgG seropositivity compared with younger participants (adjusted OR=2.80, 95% CI: 1.29–6.07; *P*=0.009). Consistent with our findings, a previous study reported a higher proportion of seropositivity among participants aged 45 yr or older than among younger individuals (24).

Consumption of fresh vegetables, sausages or hot dogs, semi-cooked or grilled meats, and contaminated water are potential sources of transmission (1, 22). The dietary habits did not differ significantly between the patient group and the controls (*P*>0.05) (Table 3). Although cats are the definitive hosts that shed oocysts, the current study reported that exposure to cats or dogs did not significantly influence parasite transmission among patients or controls. This observation is consistent with earlier research from Iraq, which reported no significant link between animal contact and toxoplasmosis prevalence (25). Similarly, previous research in Iran and Iraq showed no notable difference between those with and without cat contact (16, 26). These findings could be due to the large number of stray cats and dogs present in the participants' neighborhoods.

Results from the multiple logistic regression model after adjusting for the effects of smoking, obesity history, and history of receiving blood transfusions indicated no statistically meaningful difference in the odds of getting anti-*T. gondii* IgG seropositivity between the control and case groups. Our study assessed these factors, although no relation was found. The findings of our study differ from those reported in two other studies, which showed no association between *T. gondii* seropositivity and these factors (16, 20). However, other demographic variables, including education

level, marital status, and smoking history, were not significantly associated with *T. gondii* infection. This may reflect the need for more studies on complex interactions between these variables and infection risk. Despite the observed trends, no meaningful relationship was detected between *T. gondii* seropositivity and breast cancer after adjusting for potential confounders. The absence of significant results may be attributed to the modest number of participants and the resulting reduced statistical power. Importantly, the adjusted odds ratio remained elevated in the breast cancer group (OR=1.85, 95% CI: 0.86–4.00), indicating a possible increase in the odds of *T. gondii* seropositivity despite the absence of statistical significance. The relatively wide confidence interval suggests that the estimate was imprecise, most likely because of the limited sample size. Further studies with larger populations are needed to provide more precise estimates and to better determine the clinical significance of this association.

The present study has several limitations. In addition to the relatively small sample size and incomplete questionnaire responses from some participants, detailed clinical information on breast cancer, including tumor stage, tumor grade, treatment modalities, chemotherapy, and other immunosuppressive therapies, was not available. As these factors may influence the immune status of patients, their absence prevented subgroup analyses and should be considered when interpreting the serological findings. Although both anti-*T. gondii* IgG and IgM antibodies were evaluated, molecular confirmation by polymerase chain reaction (PCR) and IgG avidity testing was not performed. The inclusion of these complementary methods could have improved the accuracy of infection status classification and strengthened the interpretation of the serological findings. The hospital-based recruitment of participants may also have introduced selection bias, while the use of questionnaire-based data collection may have been subject to recall bias. These limitations should be taken into account when interpreting the study findings. Furthermore, because the study relied on serological markers, the findings should be interpreted with caution, as serological assays mainly identify previous (IgG) or recent (IgM) exposure and may not fully characterize the infection status.

Conclusion

Anti-*T. gondii* IgG seropositivity was observed in 48.6% of breast cancer patients and 33.9% of controls. Although the adjusted odds of seropositivity were higher among breast cancer patients (aOR=1.85, 95% CI: 0.86–4.00), this difference was not statistically significant (*P*=0.11). In contrast, age over 50 yr was significantly associated with IgG seropositivity (aOR = 2.80, 95% CI: 1.29–6.07; *P*=0.009). Overall, our findings do not provide sufficient evidence of an association between *T. gondii* seropositivity and breast cancer. Further well-designed studies with larger sample sizes and more detailed clinical data are needed to clarify this relationship.

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Conflicts of interest

The authors state that there are no conflicts of interest to disclose.

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