Seroprevalence and Risk of Toxoplasma gondii Reactivation in Pediatric Patients with Hematological Malignancies Undergoing Chemotherapy: A Case-Control Study

Babak Abdolkarimi [1], Hosein Mahmmodvand [1], Neda Beyranvand [1], Narges Naderi [1], Bardia Amidi [1]

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1 Lorestan University of Medical Sciences, Khorramabad, Iran

Correspondence to:
Bardia Amidi and Hosein Mahmmodvand
email: bardia.amidii@gmail.com; dmahmodvand@gmail.com

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SUMMARY

Background: *Toxoplasmosis, caused by Toxoplasma gondii, affects around 40% of the Iranian population and can be severe in vulnerable patients, such as those receiving chemotherapy. In this study, the titers of IgG and IgM antibodies in 92 children treated with chemotherapy have been compared with 92 matched controls. We also looked into the demographic and lifestyle factors in association with the antibody levels as a contribution to the development of improved preventive and management techniques.*

Methods: *In this case-control study conducted at Shahid Madani Hospital, Khorramabad, Iran, blood samples of both groups were tested for IgG and IgM anti-Toxoplasma gondii antibodies by ELISA. The participants were selected randomly, and demographic, clinical, and lifestyle data were obtained from structured interviews and from the hospital records. Statistical analyses were performed using SPSS software, considering p-values less than 0.05 as significant. The approval for ethics was obtained, and an informed consent was provided by the guardians.*

Results: *The results revealed that the prevalence of IgG antibodies was significantly higher in chemotherapy patients (35.9%) compared to the controls (14.1%), indicating a strong association between immunosuppression and elevated IgG levels ($p = 0.001$, $OR = 2.026$). No significant difference in IgM antibodies was found, suggesting that chemotherapy increases the risk of reactivation rather than new infections. Subgroup analysis showed that IgG positivity was more common in younger immunocompromised patients (under 10 years old). However, factors such as gender, residence, and dietary habits did not significantly affect IgG or IgM positivity. In the control group, urban residents had a higher IgG positivity rate than rural ones.*

Conclusion: *In conclusion, pediatric patients who have undergone chemotherapy are more prone to chronic infection with Toxoplasma gondii. Serological tests and prevention measures must be carried out regularly to reduce the risk of reactivation in such patients.*

Keywords: *toxoplasma, seroepidemiologic studies, hematologic neoplasms*

INTRODUCTION

Contamination of soil, water, and food is a commonly occurring route of transmission for *Toxoplasma gondii*, a protozoan parasite that can infect a wide range of hosts, including mollusks, cold-blooded and warm-blooded animals, as well as humans. Toxoplasmosis, the disease caused by this parasite, has established chronic infection in approximately one-third of the global human population (1-3). According to prior studies conducted in Iran, this parasite infects around 40% of the general population (4). The parasite can survive in the host through the formation of tissue cysts, especially in sites with minimal activity of the immune system, such as the central nervous system (5). Toxoplasmosis is primarily transmitted through the ingestion of oocysts present in contaminated food or water (6). Infection can also be acquired through the consumption of raw or undercooked meat, especially from cattle, sheep, and pigs, which contains infected tissue cysts (7). Although less common, *T. gondii* can also be transmitted through organ transplantation or blood transfusions from infected donors (8-10).

Conventional diagnosis of toxoplasmosis has generally involved the detection of parasite-specific antibodies such as IgG and IgM in patient serum, usually through serologic procedures using the enzyme-linked immunosorbent assay (ELISA) and immunofluorescence antibody assay (IFA) methods. These techniques are commonly used to confirm both recent and previous infections (11). Although toxoplasmosis is usually so mild in healthy individuals that it causes no symptoms or only a transient fever (12), it can become life-threatening in those who are immunocompromised, such as patients undergoing chemotherapy (13). Toxoplasmosis most commonly causes encephalitis, myocarditis, or pneumonitis in immunocompromised individuals (14). Furthermore, there have been instances of situations when toxoplasma has spread throughout the body. This is due to the incompetent immune responses that allow the transition from the dormant bradyzoite stage to the active, rapidly reproducing tachyzoite form, leading to the initiation of an acute infection in a patient with chronic infection (15, 16). Such may also be from re-infection or even first-time infection with the parasite. Considering the risks of toxoplasmosis in immunocompromised patients, including those receiving chemotherapy treatment, and the importance of guiding effective prevention, management, and treatment

strategies, the aim of this study was to assess and compare IgG and IgM antibody levels against *Toxoplasma gondii* in 92 children treated by chemotherapy and 92 healthy children in the control group. We have also analyzed the interrelationship between the levels of IgG and IgM antibodies and gender, age, place of residence, raw meat consumption, exposure to cats, fast food consumption, and consumption of raw milk.

METHOD

Study Design and Population

This case-control study was conducted to determine the seroprevalence of Toxoplasma gondii antibodies in pediatric patients with hematological malignancies following chemotherapy in comparison with a healthy group. Patients were selected from the Pediatric Oncology Department of Shahid Madani Hospital in Khorramabad, Iran. 92 immunosuppressed pediatric patients, aged between 1-18 years, who had confirmed hematological malignancies and received chemotherapy were studied. The controls were age and sex matched, with a population of 92 healthy individuals without any immunosuppressive or hematological diseases. This study follows the guidelines outlined in the 2007 STROBE checklist for reporting observational research in epidemiology.

The inclusion criteria for the patient group included the presence of hematological malignancy and immunosuppression due to chemotherapy, which was supported by laboratory findings including lymphopenia (peripheral blood lymphocyte count $<1500/mm^3$ or $<2000/mm^3$ in children under 6 years) and/or hypogammaglobulinemia (low serum IgG levels).

Sampling Method

The participants were selected using random sampling to minimize bias. 92 pediatric patients who fulfilled the inclusion criteria of immunosuppression were randomly selected from the department of pediatric oncology. The controls were matched for age and sex with a similar random selection.

Data Collection

All demographic, clinical, and lifestyle information was garnered from either group. The patients' clinical data included cancer type, chemotherapy history, and immunosuppressive status (determined by the test results). For the lifestyle information, dietary habits were questioned regarding the consumption of raw meat, fast food, and unpasteurized milk. Environmental exposure (such as interaction with cats) was among the lifestyle data obtained for both groups. The structured interviews were carried out with parents or guardians, and hospital records were obtained.

Blood Sample Collection and Serological Testing

Blood samples (5 cc) were collected from each participant, and a serum was isolated and stored at -20°C for analysis. Anti-Toxoplasma gondii IgG and IgM antibodies were assayed by ELISA. In the present study, ELISA/IgG and ELISA/IgM kits from Euroimmun, Germany, were used. The seropositivity was classified as positive (≥1.1 IU/ml) or negative (<0.8 IU/ml). The samples which gave results in the borderline range of 0.8-1.1 IU/mL were re-tested. To ensure the accuracy of the results, the sera were kept at -91°C until the completion of the trial.

Statistical Analysis

SPSS version 20.0 was used for statistical analysis. Paired Student's t-test compared the age values between groups. A Chi-square test was done to assess the association between Toxoplasma gondii seropositivity with demographic, clinical, and lifestyle characteristics. The odds ratios (ORs) were calculated to measure the strength of associations based on the chi-square test results. A p-value of <0.05 was considered statistically significant.

Ethical Considerations

This study was approved by the Ethics Committee of Lorestan University of Medical Sciences (IR.LUMS.REC.1397.124). An informed consent was obtained from all the participants' parents or guardians, and participation was voluntary with the option to withdraw at any time.

RESULTS

In this study, 184 participants were included, consisting of 92 patients with hematological malignancies with immunosuppression due to chemotherapy and 92 healthy controls. The demographic characteristics of the participants are presented in Table 1.

Table 1. Frequency Distribution of Demographic Characteristics Based on the Study Groups.

Variables		Cases		Controls		p-value*
		No	(%)	No	(%)	
Gender	Male	40	43.5%	47	51.1%	.303
	Female	52	56.5%	45	48.9%	
Age	10 = <	70	76.1%	69	75%	+6
	>10	22	23.9%	23	25%	
Residence	Urban	42	45.7%	41	44.6%	883
	Village	50	54.3%	51	55.4%	
Education	<Diploma	77	83.7%	72	78.3%	.349
	>Diploma	15	16.3%	20	21.7%	
Raw meat consumption	No	92	100%	89	96.7%	.321
	Yes	0	0%	3	3.3%	
Unwashed fruit consumption	No	68	73.9%	73	79.3%	.385
	Yes	24	26.1%	19	20.7%	
Ready-to-eat food consumption	No	83	90.2%	78	84.8%	.266
	Yes	9	9.8%	14	15.2%	
Cat Contact	No	88	95.7%	90	97.8 %	.341
	Yes	4	4.3%	2	2.2 %	
Agricultural Activity	No	40	43.5%	31	33.7 %	.174
	Yes	52	56.5%	61	66.3%	
Raw milk consumption	No	92	100%	90	97.8%	.156
	Yes	0	0%	2	2.2%	
Total		92	100%	92	100%	

**Chi-squared test*

There was no significant association between the disease and gender, age, residence, education, raw meat consumption, unwashed fruit consumption, ready-to-eat food consumption, cat contact, agricultural activity, or raw milk consumption.

In this study, 35.9% of immunocompromised patients tested positive for IgG antibodies, in comparison to 14.1% in the control group, with a statistically significant difference of p = 0.001. The odds of IgG positivity were 2.026 times higher in the patient group (OR = 2.026, 95% CI: 1.250–3.282). In contrast, 2.2% of the patients and 3.3% of the controls tested positive for IgM antibodies, and there was no statistically significant difference (P = 0.651, OR = 0.829, 95% CI: 0.399–1.721), suggesting no association between immunosuppression and IgM seropositivity (Table 2).

In the group of immunocompromised patients, there were no significant relationships between the positivity of IgG and gender (p = 0.776), residence (p = 0.644), cat contact (p = 0.868), or ready-to-eat food consumption (p = 0.471). However, there was a significant correlation between age and the positivity of IgG, with 40% of the patients under 10 years old testing positive versus 22.7% of the patients over 10 years old (p = 0.023). In addition, there were no significant associations between IgM positivity and gender (p = 0.105), age (p = 0.425), residence (p = 0.121), cat contact (p = 0.640), or ready-to-eat food consumption (p = 0.852) (Table 3).

In the control group, there were no significant relationships between the positivity of IgG and gender (p = 0.328), age (p = 0.842), raw meat consumption (p = 0.334), cat contact (p = 0.397), ready-to-eat food consumption (p = 0.696), or raw milk consumption (p = 0.564). However, a significant relationship was found between residence and positivity of IgG, with 26.8% of urban residents testing positive versus 3.9% of rural residents (p = 0.002). Furthermore, no significant link was found between the positivity of IgM and gender (p = 0.534), age (p = 0.312), residence (p = 0.692), raw meat consumption (p = 0.748), cat contact (p = 0.377), ready-to-eat food consumption (p = 0.212), or raw milk consumption (p = 0.794) (Table 4).

Table 2. Comparison of Anti-Toxoplasma Antibodies (IgG and IgM) in Immunocompromised Patients and Healthy Controls.

		Group		Total	p-value*	*OR (95%CI)
		Cases	Controls			
ToxoIgG	Negative: n (%)	59 (64.1%)	79 (85.9%)	138 (75.0%)	.001*	2.026 (1.250 -3.282)
	Positive: n (%)	33 (35.9%)	13 (14.1%)	46 (25.0%)		
	Total: n (%)	92 (100.0%)	92 (100.0%)	184 (100.0%)		
ToxoIgM	Negative: n (%)	90 (97.8%)	89 (96.7%)	179 (97.3%)	.651	.829 (.399-1.721)
	Positive: n (%)	2 (2.2%)	3 (3.3%)	5 (2.7%)		
	Total: n (%)	92 (100.0%)	92 (100.0%)	184 (100.0%)		

**Chi-squared test, odds ratio, significant p-value*

Table 3. Relationship between Anti-Toxoplasma Antibodies (IgG and IgM) and Characteristics of the Immunocompromised Patients.

Variables		ToxoIgG				p-value*	ToxoIgM				p-value*
		Positive		Negative			Positive		Negative		
		No	(%)	No	(%)		No	(%)	No	(%)	
Gender	Male	15	37.5	25	62.5%	.776	2	5	38	95.0%	.105
	Female	18	34.6%	34	65.4%		0	0%	52	52	
Age	10 = <	28	40	42	60	.023	2	2.9%	68	97.1%	.425
	>10	5	22.7%	17	77.3%		0	0%	22	100.0%	
Residence	Urban	14	33.3%	28	66.7%	.644	2	4.8%	40	95.2%	.121
	Village	19	38	31	62.0%		0	0%	50	100%	
Raw Meat Consumption	No	33	35.9%	59	64.1%	-	2	2.2%	90	97.8%	-
Cat Contact	No	30	36.1%	53	63.9%	.868	2	2.4%	81	97.6%	.640
	Yes	3	33.3	6	66.7%		0	0%	9	100%	
Ready-to-eat food consumption	No	16	40	24	60.0%	.471	1	2.5	39	97.5%	852
	Yes	17	32.7%	35	67.3%		1	1.9	51	98.1%	
Raw milk consumption	No	33	35.9%	59	64.1%	-	2	2.2%	90	97.8%	-

**Chi-squared test*

Table 4. Relationship between Anti-Toxoplasma Antibodies (IgG and IgM) and Characteristics of the Controls.

Variables		ToxoIgG					ToxoIgM				
		Positive		Negative		p-value*	Positive		Negative		p-value*
		No	(%)	No	(%)		No	(%)	No	(%)	
Gender	Male	5	10.6%	42	89.4%	.328	1	2.1%	46	97.9%	.534
	Female	8	17.8%	37	82.2%		2	4.4%	43	95.6%	
Age	10 = <	10	14.5%	59	85.5%	.842	3	4.3%	66	95.7%	.312
	>10	3	13%	20	87.0%		0	0%	23	100.0%	
Residence	Urban	11	26.8%	30	73.2%	.002	1	2.4%	40	97.6%	.692
	Village	2	3.9%	49	96.1%		2	3.9%	49	96.1%	
Raw Meat Consumption	No	12	13.5%	77	86.5%	.334	3	3.4%	86	96.6%	.748
	Yes	1	33.3%	2	66.7%		0	0%	3	100.0%	
Cat Contact	No	10	12.8%	68	87.2%	.397	2	2.6%	76	97.4%	.377
	Yes	3	21.4%	11	78.6%		1	7.1%	13	92.9%	
Ready-to-eat food consumption	No	5	16.1%	26	83.9%	.696	0	0%	31	100.0%	.212
	Yes	8	13.1%	53	86.9%		3	4.9%	58	95.1%	
Raw milk consumption	No	13	14.4%	77	85.6%	.564	3	3.3%	87	96.7%	.794
	Yes	0	0%	2	100%		0	0%	2	100.0%	

**Chi-squared test*

DISCUSSION

Toxoplasmosis is a common disease caused by an obligate intracellular parasite known as *Toxoplasma gondii* which infects over a third of the global population (17). Various human clinical diseases are caused by this parasite (18). While many people carry the parasite without any symptoms due to a latent infection with tissue cysts, the symptomatic infection with *T. gondii* is relatively rare. Severe cases of toxoplasmosis occur mainly in specific groups, such as fetuses and newborns who are congenitally infected and individuals with impaired immune systems (19, 20). The disease in immunocompromised patients often appears as an opportunistic infection (21) because the parasite can remain as bradyzoites in the body for the rest of your life (18). In fact, the reactivation of chronic infection leads to toxoplasmosis (22), most likely in cancer patients, patients undergoing chemotherapy, transplant recipients, and individuals with connective tissue diseases (23-25). In these individuals, toxoplasmosis can cause serious conditions like encephalitis, myocarditis (26), pneumonitis (27), or diffuse forms of infection (28), which are dependent on the host's immunological status.

Many cancer patients have weakened immune systems due to their primary illness or treatments like chemotherapy and radiotherapy. Chemotherapeutic medications attack both rapidly developing cancer cells and also healthy white blood cells, which can lead to neutropenia. This makes chemotherapy patients more vulnerable to *Toxoplasma* infection (29). According to the research, the reactivation of a latent *T. gondii* infection is more common in certain types of malignancies, particularly those affecting the brain, eye, breast, and blood (24, 30-33).

Serological testing, which is crucial in managing toxoplasmosis in immunocompromised patients, is used as a first-line test for complementary diagnosis and prevention plans. Patients at risk are identified by a positive serological test before and during bone marrow and solid organ transplant (21). In our study, we explored the prevalence of IgG and IgM antibodies of *Toxoplasma* in 92 patients with hematological malignancy and immunosuppression induced by chemotherapy and also in 92 healthy controls. IgG antibodies were significantly more common in the group of patients (35.9%) than in the group of controls (14.1%), which shows a strong link between immunosuppression and the positivity of IgG ($p = 0.001$). The odds of IgG positivity were 2.026 times higher in the patient group (OR = 2.026, 95% CI: 1.250–3.282), which indicates a strong connection between immunosuppressive conditions and increased IgG levels. Nonetheless, there was no significant difference in IgM antibodies, suggesting no relationship between immunosuppression and IgM seropositivity. Subgroup analyses showed that there was a significant link between IgG positivity and age under 10, with 40% of individuals under 10 years old testing positive compared to 22.7% of patients older than 10 years old ($p = 0.023$). This finding can be attributed to the observation that younger children frequently have a stronger and longer-lasting antibody response due to a variety of factors associated with their maturing immune systems (34). On the other hand, factors such as gender, residence, cat contact, or dietary habits did not show any significant links with the positivity of IgG or IgM in these patients. In the control group, among demographic factors, only living in urban areas was notably associated with IgG positivity (26.8% in urban vs. 3.9% in rural residents, $p = 0.002$). This can be explained

by the finding that exposure to *Toxoplasma gondii* is remarkably higher in urban areas due to the greater presence of domestic pets and small wild animals inhabiting urban environments, as well as increased interaction with contaminated surroundings (35-37).

Several studies from around the world were consistent with our findings. For example, a study by Alim et al. looked at cancer patients between the ages of 18 and 80 and found that IgG was positive in 60.0% of the cancer patients undergoing chemotherapy in comparison to the control group (27.0%) (38). Hosseini SA et al. studied cancer patients who were receiving chemotherapy in northern Iran and reported that 75.4% of patients were positive for IgG antibodies, 2.57% tested positive for IgM, and 5.43% had detectable *T. gondii* DNA (39). Moreover, Mostafa et al. reported the highest IgG positivity rates (80%) in patients with breast cancer and bone carcinoma (40). Ali MI et al. explored the seroprevalence of *Toxoplasma gondii* in cancer patients who were receiving chemotherapy. They reported that 66.7% of the cancer group tested positive for IgG and 9.2% for IgM compared to 33.3% and 6.7% in the control group ($p < 0.001$, $OR = 4$). Furthermore, they declared that the patients with hematological malignancies had increased the IgG positivity rates in comparison to the patients with solid tumors (40% vs. 26.7%) (29).

In addition, several studies have found that infection with *Toxoplasma gondii* can occur in patients receiving hematopoietic stem cell transplantation (HSCT) (41-43). Toxoplasmosis in these patients is a medical emergency, and if it is left untreated, it can be 100% fatal (44). The infection is most common in allogenic transplant recipients, though it has also been seen in autologous transplant patients (45-47). It is recommended that allogeneic HSCT recipients and their donors, as well as all the recipients of autologous HSCT, be screened with the IgG and IgM antibodies of *Toxoplasma* before the transplant (44). Around 90% of patients show positive results for these antibodies before the procedure, indicating a reactivation of latent cysts (48, 49). The disease mostly occurs during the first 6 months following the transplant, but in some cases, the onset of the condition may be delayed in the patients who experience graft-versus-host disease (GVHD) and need immunosuppressive treatment (50, 51). Preventing toxoplasmosis in HSCT recipients with seropositive *T. gondii* involves the administration of trimethoprim-sulfamethoxazole (TMPSMX) for 6 months or until the patient is no longer immunosuppressed, whichever is longer. Those who have not received prophylaxis or have been treated with other medications than TMP-SMX should be screened weekly by PCR. If any symptoms like fever, encephalopathy, altered mental status, seizures, and pneumonia appear, the patient should be treated with oral pyrimethamine, sulfadiazine, and leucovorin for at least 6 weeks and also receive secondary prophylaxis (44).

Limitations

One limitation of this study was the small sample size, which may have affected the findings. We also faced challenges in reaching materials and equipment, which limited some features of the research.

CONCLUSION

This study discloses the increased risk of *T. gondii* infection in pediatric patients under chemotherapy for hematologic malignancies. The likelihood that immunocompromised children develop chronic *T. gondii* infections is higher than in immunocompetent hosts, underscoring the imperative nature of enhanced awareness and surveillance in this population. Although the rate of chronic infections was higher, it did not show a significant increase in acute infection; thus, it may mean that immunosuppression does not always cause new infections but acts as an additional factor for the risk of reactivation. Key clinical and lifestyle factors, such as gender, residence, and contact with animals, did not relate significantly with the status of infection; therefore, the risk is more related to being immunocompromised than those factors themselves. Younger children, especially below ten years of age, were found to be more susceptible to chronic infection; this thus brings up the need for targeted screening and prevention interventions in this age group. These findings stress routine serological testing and preventive therapy in pediatric cancer patients for a reduced risk from reactivated toxoplasmosis. Indeed, proactive care can prevent major problems and improve outcomes in this population at risk.

Author Contributions

B.A. and H.M. contributed to the conception and design of the study, as well as the acquisition, analysis, and interpretation of data. B.A., N.N., and B.Am. were responsible for drafting the manuscript and critically revising it for important intellectual content. All authors reviewed and approved the final manuscript and agreed to be accountable for all aspects of the work, ensuring the accuracy and integrity of the study.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article. No financial, personal, or other relationships that could be perceived as affecting the neutrality or objectivity of the work exist.

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