Toxoplasma gondii infection in cancer patients in Guilan, Iran: prevalence and risk of acquired or reactivation of latent Toxoplasmosis

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Abstract

Introduction: *Toxoplasma gondii (T. gondii)* infection in immune-compromised individuals, including malignant patients under chemotherapy, can cause severe diseases as encephalitis or systemic infections. There is no study on toxoplasmosis in cancer patients in Guilan Province.This study was designed to determine toxoplasmosis IgM and IgG antibodies in cancer patients and to compare it to normal population in the north of Iran, Guilan.

Materials and methods: The present case control study is the first study on the prevalence of toxoplasmosis in cancer patients referred to an educational hospital in Rasht- Guilan (North of Iran), from July 2017 to January 2018. The range of the participants' age was 14–88 years, of whom 52.6% and 47.4% were male and female, respectively. Blood samples were collected from 150 immuno-competent individuals as a control group and 150 cancer patients. The antibodies (IgG/IgM) were measured by the ELISA method using available kits (ToxoIgG and ToxoIgM Euroimmun).

Results: The overall sero-prevalence of toxoplasmosis among the participants was 96 (64%) and 97/150 (64.7%) in cancer patients and controls, respectively. In patients, 94 (62.6 %%) were seropositive for only IgG, 1 (0.66%) for only IgM, and 1 (0.66%) for both IgG and IgM. In the control group, 1/150(0.66%) had IgG and IgM positive and 96/150 (64 %) were IgG positive and IgM negative that considered acute and chronic toxoplasmosis, respectively. Two out of 150 control groups were IgG and IgM borderline, respectively. This difference was not statistically significant for IgG (P= 0.8) and IgM (P=0.85) among the patients and also the control groups. Sero-prevalence of *T. gondii* infection varied significantly with age, sex, residence, cat at home, and educational level.

Conclusion: The data showed that a high percent of patients and control groups were susceptible to re-activation of latent toxoplasmosis. But In immune-compromised patients, reactivation of latent toxoplasmosis could even leads to lethal encephalitis. Therefore, the screening of toxoplasmosis should be considered more routinely in cancer patients.

Keywords: *Toxoplasma gondii, Cancer, Toxoplasmosis*

Introduction

Toxoplasma gondii (T. gondii) is a protozoan parasite infecting many animal species in the world. Toxoplasmosis in humans is largely asymptomatic, but it could cause various clinical pictures and

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even death, particularly in immune-compromised patients. The disease can be due to acquired infection or reactivation of a latent infection (1). The re-activation of *T. gondii* infection in patients with suppressed immune system, such as individuals with AIDS and malignant patients under chemotherapy or radiotherapy, can cause encephalitis (1-4). Administration of chemotherapy and radiotherapy in patients with cancer causes a decrease in T lymphocyte count and function disorder and a decrease in response to hapten in B lymphocytes (1). Generally, depending on many factors, such as sanitation levels, temperature, humidity, and contact with soil, cat and domestic animals, toxoplasmosis sero-prevalence varies from 0% to 95% and (5, 6). The prevalence of toxoplasmosis among the general population in Iran has been estimated to be 39.3% (7). This figure in the north of Iran rises up to 74.6% (8). In a previous study the parasite's DNA was detected also in river waters of Iran (9).

There is no study on toxoplasmosis in cancer patients in Guilan Province. Therefore, this study was designed to determine toxoplasmosis using the ELISA test to detect IgM and IgG antibodies in cancer patients and to compare it to normal population in the north of Iran, Guilan. It could increase our information about toxoplasmosis in cancer patients, which could be useful for better treatment and care of these patients in Guilan Province.

Materials and methods

It is known that the sero-prevalence of *T. gondii* infection varies depending on the climate conditions in communities, i.e. a low sero-prevalence in dry and high sero-prevalence in humid regions (10). This case-control study was conducted on cancer patients referring to the Clinical Oncology Department of Razi Hospital, Guilan University of Medical Sciences, North of Iran, from July 2017 to January 2018. Guilan has a humid subtropical climate with the heaviest rainfall in Iran reaching as high as 1,900 mm but generally around 1,400 mm.

The sample size was calculated with the reference sero-prevalence of 45% (11), a confidence level of 95% (Z=1.96, d=0.08 and P=0.45). One hundred and fifty (79 men, 71 women) cancer patients suffered from different types of malignancies and 150 healthy volunteers as control (74 men, 76 women) were enrolled in the study to assess the sero-prevalence of anti-*T. gondii* antibodies. The age of the cancer patient groups was 14–88 and 27–64 in the control group. All the participants were provided with information about the impacts of toxoplasmosis on human health, particularly immune-compromised patients, and the benefits of participating in the study to develop public health measures. Questionnaires obtained information about demographics and epidemiologic data (Table 1).

The blood samples were taken from 150 cancer patients, as well as 150 immune-competent individuals as the control group. Two ml of the blood sample was collected without EDTA anti-coagulant. All the tubes were centrifuged at 3500-4000 rpm for five minutes. All the collected sera were kept frozen at −20 °C until serological analysis. The ELISA method used to detect anti-*T. gondii* IgG and IgM antibodies with a commercially available enzyme immunoassay kit (Toxo IgG and Toxo IgM Euroimmun). Anti-*T. gondii* IgG antibody levels were expressed as International Units (IU)/ ml). The anti-*T. gondii* IgM cutoff values with absorbance levels < 0.9 were considered negative, 0.9 - 1.1 was assumed to be borderline, and > 1.1 was considered positive. All the assays were performed following the manufacturer's instructions and included positive and negative controls in each run.

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Table 1 Socio - demographic characteristics of cancer patients and sero-prevalence of *Toxoplasma gondii* infection.

Characteristic	Number of tested subjects	Prevalence of *T. gondii* infection		P value
Age groups (years)		Number	%	0.005
<30	15	5	33.3	
31-50	43	26	60.5	
51-70	71	45	64.3	
>71	21	19	90.5	
	150			
Sex				0.013
Male	79	57	73.1	
Female	71	38	53.5	
	150			
Residence				0.33
Urban	106	65		
Rural	44	30		
Educational Level				0.026
Uneducated	40	33	84.6	
Primary school	26	16	61.5	
Meddle school	23	13	56.5	
High school	45	23	51.1	
University	16	10	62.5	
Cat at home				0.036
yes	55	41	74.5	
No	95	54	57.4	
Consumption of raw vegetable				0.768
Yes	108	69	63.8	
No	42	26	61.9	
Sessions of chemotherapy				0.332
Less than 4	62	39	62.9	
5-10	55	35	63.6	
11-20	24	13	54.2	
More than 21	9	8	88.9	
Type of Cancer				0.082
Solid	105	71	67.6	
Diffuse	45	24	53.3	

To interpret quantitative results in this study, according to the manufacturer's recommendations, IgG absorbance levels < 9 IU/ml were negative, 9 - 11 was borderline, and > 11 was considered positive. The positive IgG and negative IgM test in a participant were interpreted as a latent infection. The positive IgG test and positive IgM test in a participant were interpreted as probability of a recent or acute infection (10). We again re-checked IgG^- and IgM+ false positive samples for final confirmation.

The relationship between cancer type, age, gender, educational level, contact with cat, residency, consumption of raw vegetable, session of chemotherapy and anti-*Toxoplasma* antibody sero-prevalence was investigated. Table 1 shows the patients' general demographic characteristics (such as age, gender, residency, and educational level), risk factors for toxoplasmosis (cat at home, consumption of raw vegetable,

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sessions of chemotherapy), and clinical findings, including type of cancer. The study was approved by the Ethics Committee of Guilan University of Medical Sciences with ethics number of IR.GUMS.REC.1396.164

Statistical analysis

The data were analyzed using the SPSS software (Version 18). The confidence interval was considered $P < 0.05$.

Results

In the present study, 96 out of 150 (64%) patients were seropositive for anti-*Toxoplasma* antibodies. Sero-prevalence of toxoplasmosis was found higher in patients with solid (67.6%) than diffuse cancer (53.5%), but no statistically significant difference was seen between these groups (p=0.08). The overall sero-prevalence of toxoplasmosis among cancer patients was observed in 96 (64%) participants, of whom 94 (62.6 %%) were sero-positive for only IgG, 1 (0.66%) for only IgM, and 1 (0.66%) for both IgG and IgM. In the control group, 97 out of 150 healthy individuals (64.7 %) had positive results in the *Toxoplasma* IgM and IgG serology. In the control group, 1/150(0.66%) had IgG and IgM positive and 96/150 (64 %) were IgG positive and IgM negative that considered acute and chronic toxoplasmosis, respectively. In both groups (patients and control), 2 cases (1.33%) had equivocal IgG and IgM results. Table 1 presents the frequencies of IgG antibodies against *T. gondii*. The *T. gondii* sero-preevalences was not statistically significant for IgG ($\chi 2 = 0.44$, P= 0.8) and IgM ($\chi 2 = 0.34$, P=0.85) between the patient and control groups. The range of the participants' age was 14–88 years, of whom 52.6% and 47.4% were male and female, respectively. There was a significant difference between the positive rate of *Toxo*-antibodies and increase of age in both patient and control groups (P=0.005). *Toxoplasma* sero-pervalence had the highest rate at the age over 71 years (90.5%), and the lowest rate at the age under 30 years (33.3%). Anti-*Toxoplasma* antibodies were also found higher in males (73.1%) than in females (53.5%), and the difference was statistically significant (P=0.013). The results indicate that *T. gondii* sero-prevalence is significantly associated with education level (uneducated, primary school, middle school, high school and university); individuals with no education had the highest sero-prevalence of *T. gondii* exposure (84.6%) (p=0.026). In the present study, there was a significant relationship (P=0.036) between the prevalence rate and having cat at home (P=0.036). Sero-positivity rate of *T. gondii* was also higher in individuals consuming raw vegetables (P= 0.768), and it was higher among urban dwellers (65%) than that of rural (30%); both differences were not statistically significant (P = 0.768, P=0.33, respectively) (Table 1). Moreover, there was not a correlation between the chemotherapy sessions that may affect the patients' immune-competency state and rising antibodies titer (P=0.3).

Discussion

Complications of toxoplasmosis in immune-compromised patients are usually due to reactivation of latent infection. It could even leads to lethal encephalitis (12). In the present study, 62.6 % of the patients and 64% of the control group were confirmed to be positive for *Toxoplasma* IgG antibodies. The overall sero-prevalence of toxoplasmosis among cancer patients was observed in 96 (64%). One out of 150 in both control and patient groups had positive IgG and IgM results. This difference was not statistically significant for IgG ($\chi 2 = 0.44$, P= 0.8) and IgM ($\chi 2 = 0.34$, P=0.8). In a systematic review and meta-analysis study on immune-compromised patients in Iran, the sero-prevalence rate of toxoplasmosis among cancer patients was 45.06% (11). The sero-prevalence values of

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toxoplasmosis reported in cancer patients in Turkey in two studies were 63% (13) and 60.0% (14) that are relatively close to our results. In contrast to our results, the prevalence of toxoplasmosis among cancer patients in Cairo was 20% (15) and China 16.72% (16), respectively. Compared to the above-mentioned studies, this study revealed that the prevalence rate of toxoplasmosis in both groups (patients and control) was higher than that of the sero-prevalence rates reported in other parts of Iran and some studies in the world (11, 15, 17-19). The high sero-positivity rate in this study comparing to other studies may be due to suitable humid climate conditions and the high possibility of exposure to contamination sources (10). This high prevalence of IgG^+ in cancer patients shows a potential risk to re-activate latent toxoplasmosis in patients requiring frequent chemotherapy. In addition to the high prevalence of IgG antibody, IgM anti-*Toxoplasma* antibody was detected in both cancer and control groups. Therefore, it seems reasonable to deliver adequate care to prevent a possible acute toxoplasmosis, especially in immune-compromised patients.

Some studies demonstrated that there was a possible association between *T. gondii* infection and some forms of cancer (17, 20). The prevalence of *T. gondii* in the present study was higher in patients having solid organ tumors (68.3%) than those with hematological malignancies (53.3%); however, this difference was not statistically significant (p=0.082) (Table 1). A significant increase was observed between sero-positivity rate and age in both patient and control groups (P< 0.005); this finding is in agreement with other studies in Brazil (21), Malaysia (22), Iran (23), Japan (24) and Ireland (25). Our results show, the highest rates of toxoplasmosis sero-prevalence were observed in age groups over 71 (90.5%) years old. This tendency reflects the increased possibility of exposure to toxoplasmosis during a person's lifetime. The prevalence of *T. gondii* was significantly higher in males (73.1%) than in females (53.5%) (p<0.013) (Table 1). This finding is consistent with some studies (7, 26-29). This may be due to the increased risk of exposure of males due to more outdoor activities and/or the habit of eating outdoor foods, particularly consumption of traditional kebab food, which is very popular in the study area. The overall sero-prevalence of toxoplasmosis was higher in urban (65%) than in rural dwellers (30%) (p=0.33) (Table 1). A study in Burkina Faso identified that living in urban area was among the possible risk factors associated with *T. gondii* infection (30). In recent years, the lifestyle has changed, and an ever-increasing rise in health facilities and public health promotion in rural areas have reduced the prevalence of many infections transmitted through animals to humans. In Present study, a significant association between *T. gondii* sero-prevalence and educational level was observed, and individuals with no education showed the highest sero-prevalence of *T. gondii* exposure (84.6%) (p=0.026) (Table 1). This finding is consistent with some reports from the Netherlands (31), the United States (32), and Ethiopia (33). The role of cat as the main cause of *Toxoplasma* transmission is demonstrated. In the present study, there was a significant relationship (p=0.036) between the prevalence rate and having a cat at home (Table 1). Fresh vegetables and water are potential source of parasite contamination (34). In the present study, no significant difference between the consumption of raw vegetables and the frequency of toxoplasmosis found (p=0.768) (Table 1). Moreover, in the present study, *T. gondii* sero-prevalence was not significantly influenced by the number of the chemotherapy sessions for cancer patients (P=0.3). Compared to other studies, the epidemiological findings of present study, revealed a higher rate of infection with *T. gondii* in cancer patients.

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Therefore, we recommend cancer patients be regularly monitored to avoid the risk of reactivation of chronic toxoplasmosis. Furthermore, to prevent acute toxoplasmosis, *T. gondii* sero-negative patients must be aware of the routes of toxoplasmosis transmission. This could be useful to reduce healthcare expenditure.

Conclusions

In immune-compromised patients, reactivation of latent toxoplasmosis could even lead to lethal encephalitis. A considerable proportion of cancer patients in this study were potentially susceptible to reactivation of latent toxoplasmosis. To implement early prophylaxes or other control interventions, monitoring the *Toxoplasma* antibody status in cancer patients is recommended.

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