Original Article

Investigation of the Prevalence of Toxoplasmosis in Patients with Malignancies, Southwest of Iran

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ABSTRACT

Toxoplasmosis, a parasitic disease, has the capacity to infect humans, other warm-blooded animals, and cats. Individuals with compromised immune systems, including cancer patients receiving chemotherapy, individuals with AIDS, transplant recipients, and hemodialysis patients, are at an increased risk of contracting toxoplasmosis. The challenge in diagnosing toxoplasmosis in such cases stems from the similarity of certain symptoms to those of cancer or chemotherapy-related complications. Consequently, the ELISA test is employed to ascertain the presence of the infection and to determine the quantity of antibodies in the patient. A total of 90 cancer patients who had been admitted to the hospital and were undergoing chemotherapy were included in the study. Blood samples were collected from these patients, who were at various stages of chemotherapy. The anti-toxoplasma antibody titer was subsequently determined using the ELISA method, and the data were analyzed using SPSS version 23 software. In this study, 50 (55.6%) of the subjects were female and 40 (44.4%) were male. All samples were found to be negative for IgM antibody titers, while 50 (55.6%) were positive for IgG antibodies. In patients with positive test results, the most common clinical symptoms were lethargy and anorexia. While anti-toxoplasma IgG antibodies exhibited a higher prevalence among male subjects, no statistically significant discrepancy was observed between gender and infection rates. Furthermore, in comparison with individuals who have not received chemotherapy, those with a history of chemotherapy exhibited elevated levels of anti-toxoplasma IgG antibodies. The level of anti-toxoplasma IgG in malignant patients hospitalized in this hospital was high, but statistical analysis showed a significant difference between the prevalence of toxoplasma and the type of cancer. Individuals diagnosed with cancer face an elevated risk of developing severe toxoplasmosis and its associated consequences, attributable to the high incidence of T. gondii. Consequently, oncologists must recognize this grave medical condition as necessitating prompt attention.

Keywords: *Toxoplasmosis,* Complications, *Diagnosis,* Epidemiology, Prevention and Control.

1. Introduction

It is estimated that one-third of the global population is infected with the obligate intracellular eukaryotic opportunistic pathogen Toxoplasma gondii, which has the capacity to infiltrate and proliferate in a variety of host organs (1). Toxoplasmosis is a zoonosis illness that affects cats as the sole definitive host, with humans and other warm-blooded animals serving as intermediate hosts (2). The protozoan can spread via the placenta from mother to foetus, through the use of oocyst-contaminated water and food, through the consumption of undercooked meat, through organ transplants, and through blood transfusions (3). In the majority of hosts with a robust immune system, innate immune responses are responsible for the control of tachyzoite growth during the acute phase of infection. During this period, tachyzoites transform into bradyzoites and subsequently form cysts within the brain, eye, and muscle tissues. These cysts persist within the host organism for the remainder of its lifespan, either as a consequence of an immune response or physiological stress (4, 5). As demonstrated by research, individuals afflicted with cancer who are undergoing chemotherapy, those with a compromised immune system, patients living with AIDS, transplant recipients, and haemodialysis patients are predisposed to a heightened risk of contracting toxoplasmosis (6–9). Research has indicated that individuals diagnosed with certain forms of cancer, including eye, brain, blood, and breast malignancies, are more susceptible to reactivation of latent Toxoplasma gondii infection (10). The immunological response to a toxoplasmosis infection is contingent on genetic diversity and immune system composition. It is imperative to emphasize that the parasite has the potential to disseminate to all tissues, particularly the central nervous system and the placenta (11). In individuals with a compromised immune system, the illness manifests as fever, muscular discomfort, eye damage, CNS abnormalities, etc., and may cause severe damage, including encephalopathy and meningoencephalitis (2). The prevalence of toxoplasmosis in Iran has been estimated to be 39.3% in the general population, 51.01% in immunocompromised patients, and 44% in pregnant women (12). The primary objective of this research was to examine toxoplasmosis in chemotherapy-treated cancer patients referred to Bagai 2 Hospital in Ahvaz, Iran, in 2001, and to determine whether or not this illness is associated with specific forms of cancer. It is imperative to note that the confidentiality of the participants' information and all other ethical criteria are strictly adhered to throughout the course of this study.

2. Materials and Methods

2.1. Data Collecting

The present study constitutes an analytical cross-sectional investigation into the prevalence of toxoplasmosis in cancer patients referred to the oncology department of Baqaei 2 Hospital in Ahvaz for chemotherapy in 2014. As stated in the study conducted by Hosseini et al. (13), 90 patients with various malignancies (leukemia, lymphoma, lung cancer, breast cancer, uterine cancer, and colon cancer) were included in the study as available samples. The study had a confidence level of 95% and an error margin of 9%.

2.2. Blood Sampling

Following the acquisition of informed consent, 3 cc of blood was drawn from each patient, and the samples were subjected to centrifugation for 5 minutes at 3000 rpm to extract the serum. Subsequently, the serum was transferred to a tube that had been newly labelled. An enzyme-linked immunosorbent assay (ELISA) was employed to ascertain the presence of IgM and IgG antibodies against toxoplasmosis in the serum sample. The sera were diluted with a 1:100 dilution, as directed by the manufacturer (Pishtaz Medicine of Iran). Subsequently, the samples were subjected to measurement of optical absorption at 450 and 630 nm using an ELISA reader. Interpretation of IgG results: In accordance with the instructions provided by the kit's manufacturer, individuals exhibiting antibody levels that exceed 11 IU/mI are designated as positive, while those registering below 9 IU/mI are classified as negative. Values ranging from 9 to 11 IU/mI are considered to be of uncertain significance. The objective of this study is to report the results of the amount of IgM antibody, for which an index cut-off value is first obtained by dividing the optical absorbance of the sample. Should the value obtained be higher than 1.1, it is considered positive; if the value is lower than 0.9, it is considered negative; and if the value falls between 0.9 and 1.1, it is considered suspicious. A researcher's checklist was utilised in order to collect information. This checklist included demographic information and possible risk factors related to Toxoplasma gondii, such as age, gender, type of malignancy, clinical symptoms, history of chemotherapy and duration of chemotherapy.

2.3. Data Analysis

The SPSS version 23 software was utilized throughout the process of statistical analysis. In order to ascertain whether the variables in the research followed a normal distribution, the Kolmogorov-Smirnov test was performed. In the descriptive statistics, frequency and percentage played important roles. The number of individuals who tested positive for the serological test (anti-T. gondii IgG) was

divided by the total number of individuals who underwent testing for the desired type of cancer. This enabled researcher to determine the prevalence of toxoplasma infection in cancer patients according to the type of cancer. This enabled the calculation of the prevalence of toxoplasma infection in cancer patients. The Chi-square test and the independent t-test were utilised to investigate the existence of a correlation between the qualitative and quantitative factors. The quantitative and qualitative variables were examined using an independent t-test. In order to evaluate the connection between toxoplasmosis and possible risk variables, a multivariable logistic regression model was used. The odds ratios were computed using the odds ratio method, and the confidence intervals were set at 95%. (CI). In instances where the p value exceeded 0.05, statistical significance was presumed.

3. Results

The present study encompasses a total of 90 patients diagnosed with malignancies, comprising 50 female patients (55.6%) and 40 male patients (44.4%). It was determined that all samples were negative for IgM antibody titers. However, 50 samples were positive for IgG antibodies, representing 55.6% of all samples. Fifty out of ninety patients (55.6%) were found to be positive for anti-toxoplasma antibodies. The following types of cancer were observed among the patients: A total of 16 cases of lymphoma, 24 cases of leukemia, 15 cases of colon cancer, 9 cases of stomach cancer, 9 cases of breast cancer, and 17 other malignancies (including lung, ovary, sarcoma and liver) were documented. Although toxoplasmosis was more prevalent in patients with leukemia (70.8%), no significant difference was observed in the incidence of toxoplasmosis among patients with other types of cancer (P = 0.576). The presence of symptoms similar to those of cancer or chemotherapy-related problems can make it challenging to diagnose toxoplasmosis. In instances where positive IgG titers were identified, a higher prevalence of symptoms such as muscle pain, headaches, physical weakness, skin rashes, anorexia, and fever and chills was observed. Notably, anorexia emerged as the most prevalent clinical symptom among the study participants (36.7%), and in those with a positive test result, lethargy and anorexia were the most commonly reported symptoms. With regard to the risk variables, no statistically significant differences were identified between the demographic features of the analysed populations (Table 1). The findings of a multivariate logistic regression study indicate that there is no statistically significant difference between the prevalence of T. gondii in serum based on a person's gender (OR = 1.89, 95% CI: 0.61 to 5.79, p = 0.265). Notwithstanding this fact, the findings of the serological tests demonstrated that the prevalence of anti-Toxoplasma IgG antibodies was considerably higher in men than in women. The patients participating in this research study ranged in age from 16 to 81 years, thus encompassing the specific age group under investigation. A comparison of the prevalence of anti-Toxoplasma IgG antibodies between age groups has revealed a significant increase in the prevalence among individuals between the ages of 51 and 60, as well as between 61 and 70, in comparison to younger age groups. Furthermore, the likelihood of infection with Toxoplasma increased in individuals of these ages relative to those of younger ages. The results of the serological tests demonstrated that individuals with a history of chemotherapy were more likely to have anti-toxoplasma IgG antibodies compared to those with cancer who had not undergone chemotherapy. The statistical analysis further revealed a significant difference between the seroprevalence of T. gondii in cancer patients with a history of chemotherapy and those without such a history (OR = 6.52, 95% CI: 1.31 to 32.33, p = 0.022). Patients who had undergone more than 11 chemotherapy sessions were shown to have a greater seroprevalence of T. gondii antibodies in their systems. Indeed, the prevalence of T. gondii exhibited a marked increase in cases where chemotherapy was administered over extended periods. Despite the absence of a statistically significant correlation between the number of chemotherapy sessions and toxoplasma infection, the probability of testing positive for T. gondii does increase proportionately with the number of chemotherapy sessions received. The results of the serological analysis of this study indicate a significant frequency of anti-toxoplasma IgG in cancer patients who were hospitalized at this facility. Despite the elevated risk of cancer patients contracting toxoplasmosis, statistical analysis revealed no discernible relationship between the prevalence of toxoplasma and the specific kind of cancer (Table 1).

4. Discussion

It is hypothesized that T. gondii is an oncogenic pathogen, and that it plays a role in the induction and progression of malignant diseases. A number of theories have been postulated to explain this phenomenon, including the prevention of apoptosis and the enhancement of the mobility of dendritic cells and macrophages (12). Mustafa et al. conducted a study in Egypt in 2016 under the title of the relationship between toxoplasmosis and various types of human tumors. The study involved 156 patients with

Table 1. Demographic Characteristics and Risk Factors of *toxoplasma gondii* among cancer patients.

Variable		No. of samples, n (%)	No. of positives, n (%)	P.value
Sex	Women	50 (55.6)	27 (54.0)	0.832*
	Men	40 (44.4)	23 (57.5)	
Age	≤ 30	7 (7.8)	3 (42.1)	0.840*
	31 - 40	19 (21.1)	11 (57.9)	
	41 - 50	20 (20.0)	8 (44.4)	
	51 - 60	19 (21.1)	12 (63.2)	
	61 - 70	18 (20.0)	11 (61.1)	
	≥ 71	9 (10.0)	5 (55.6)	
History of chemotherapy	No	77 (85.6)	46 (59.7)	0.071*
	Yes	13 (14.4)	4 (30.8)	
Number of chemotherapy sessions	1 - 3	36 (40.0)	21 (58.3)	0.482*
	4 - 7	27 (30.0)	12 (44.4)	
	8 – 11	14 (15.6)	8 (57.1)	
	> 11	13 (14.4)	9 (69.2)	
Types of Cancer	Colon	15 (16.7)	8 (53.3)	0.576*
	Breast	9 (10.0)	5 (55.6)	
	Stomach	9 (10.0)	5 (55.6)	
	Leukemia	24 (26.7)	17 (70.8)	
	Lymphoma	16 (17.8)	6 (37.5)	
	Other	17 (18.9)	9 (52.9)	
Clinical signs	Fever	28 (31.1)	14 (28.0)	0.757**
	Chills	26 (28.9)	14 (28.0)	0.956**
	Fatigue	21 (23.3)	16 (32.0)	0.111**
	Nausea	19 (21.1)	9 (18.0)	0.625**
	Narcosis	31 (34.4)	21 (42.0)	0.312**
	Muscular pain	26 (28.9)	14 (28.0)	0.963**
	Anorexia	33 (36.7)	21 (42.0)	0.393**
	Headache	18 (20.0)	12 (24.0)	0.982**

*Chi. Square Test, **Independent samples Test

various cancers, and the researchers used an immunoassay method to determine the titer of IgG and IgM antibodies against toxoplasma. The investigation focused on the correlation between T. gondii infection and the development of tumors, with the research findings suggesting a potential link between toxoplasmosis and the development of specific types of cancers. The regulation of the macrophage proteome in mice infected with this illness has been used in research on the function of Toxoplasma as a significant pathogen connected to the incidence of glioma and meningioma (14). In the present study, the samples were found to be negative for IgM antibody titer (50/90, or 55.6%) and were predominantly female (40/90, or 44.4%), although 50/90 (55.6%) were found to be positive for IgG antibodies. This is a result that is highly comparable to that of researchers in Turkey. (63%)(15), despite the higher antibody titers observed in China (16.7%) (16) and Egypt (20%) (17). In the present study, the prevalence of IgG anti-toxoplasma antibodies was found to be higher in men than in women. However, no statistically significant correlation was identified between gender and the serum prevalence of T. gondii (OR = 1.89, 95% CI: 0.61 to 5.79, p = 0.265). This was determined by examining the multivariable logistic regression analysis. A review study conducted in Pakistan by Shoukat et al. revealed that males exhibited a higher prevalence of toxoplasmosis compared to females. The researchers also identified a robust correlation between gender and infection (18). The findings of this study

indicated that the prevalence of anti-toxoplasma IgG antibodies was greater in the age groups of 51-60 and 61-70 than in the younger age groups. Furthermore, the probability of infection with Toxoplasma was observed to be elevated in these specific age demographics in comparison to other age groups. The present study revealed that the frequency of toxoplasma antibodies in patients with cancer was very high (55.6%), although statistical analysis did not reveal a significant correlation between toxoplasma prevalence and cancer type. In a 2015 study conducted by Kalantari et al., the relationship between toxoplasmosis and breast cancer in Iran was investigated. The study comprised 66 women with breast cancer, of whom 29 had recently been diagnosed with the disease and 37 were undergoing treatment and regular examinations. In addition, 60 healthy women without a history of cancer were used as a control group. The results obtained revealed that immunoglobulin (IgG) levels were significantly higher in breast cancer patients (86.4%) and cancer patient controls (100%) (19). Mustafa et al. found associations between toxoplasmosis and many forms of cancer, including breast cancer, squamous cell carcinoma in bone, brain tumours (glioblastoma and astrocytoma), liver tumors, bladder cancer, and benign tumours. A significant relationship was observed in uterus cancer (14). The findings of a study (20) suggest that long-term host defensive responses generated by recurrent infections may raise the prevalence of cancer by promoting inflammation and boosting cell mutations. The collapse of cell barriers and the subsequent appearance of oncogene mutations following infection with intracellular organisms may be responsible for the development of cancer (21). There is evidence to suggest that toxoplasmosis has the potential to cause cancer in humans (22). It has been demonstrated that Trypanosoma cruzi (23), Toxoplasma gondii (24), Acanthamoeba castellanii (25), Echinococcus granulosus (26), and Trichinella spiralis (27) have the capacity to induce antitumor activity against various types of cancer. However, a retrospective study conducted in Cyprus demonstrated that Echinococcus may elevate the risk of cancer in patients (28). It has been documented in several studies that there exists an antigenic similarity between lung carcinoma and hydatid cyst fluid (29). In order to minimize the mortality of malignant illnesses brought on by the complications of toxoplasmosis recurrence, it is proposed that investigations comparable to the current investigation be conducted. Whilst the contraction of parasitic diseases is widely regarded as a potentially dangerous condition, recent studies have indicated that these infections may not always be opportunistic. It is important to note that positive IgG and negative IgM results might be interpreted as a latent toxoplasmosis infection. Consequently, the findings of this research are of significant assistance in minimizing difficulties caused by the return of toxoplasmosis in cancer patients.

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Authors' Contribution

R S K, M E R, A H, were the major contributors to conceptualizing and formulating the research question and designing the study.

R S K, T V were the leader of the research and project team.

M E R, A F K and MO E R, M A N, collected and analyzed the data.

R S K and A F K, wrote the first draft of the manuscript.

A H, and R S K, critically studied and appraised the first draft.

A F K and R S K, revised and developed the first draft based on a critical appraisal of their colleagues. All authors commented on the modified draft, and the final version of the manuscript was prepared. Finally, all authors approved the final manuscript.

Ethics

This study was approved by the ethics code IR.AJUMS.REC.1401.309, at Jundishapur University of Ahvaz.

Conflict of Interest

The present research does not involve any conflict of interest.

Data Availability

All the data of this research and the right to publish it are at the disposal of this journal.

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