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Seroprevalence of *Toxoplasma gondii* in Cancer Patients Admitted to Hospitals of the Royal Medical Services in Jordan

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Abstract

Several seroprevalence studies on *Toxoplasma gondii* were conducted in Jordan, but none of them investigated its seroprevalence among patients who suffer from different types of cancer. Thus, the present cross-sectional study on two-hundred Jordanian cancer patients and ninety healthy normal subjects (controls) was conducted in order to detect the presence of anti-*T. gondii* antibodies (IgG and IgM) serologically using commercial ELISA. Out of the two-hundred recruited cancer patients, anti-*T. gondii* IgG and IgM antibodies were detected in seventy-nine and five patients (39.5 % and 2.5 %) respectively, and four patients (2 %) had both IgG and IgM antibodies. In the control group, eleven cases (12.2 %) and only one case (1.1 %) were tested positive for IgG and IgM antibodies respectively. In addition, only one case from the control group (1.1 %) had both IgG and IgM antibodies against *T. gondii*. The difference in the IgG seropositivity rates between the patients and the control group is statistically significant (P <0.05) indicating that these patients are at a higher risk to acquire toxoplasmosis. The seroprevalence of *T. gondii* IgG and IgM antibodies increased significantly with age in the control group only. More studies are required in order to investigate the possibility of dissemination, and the severity of toxoplasmosis among cancer patients especially the IgM-positive patients which indicate a current infection.

Keywords: Toxoplasmosis, Seroprevalence, Cancer, ELISA, IgG, IgM, Chemotherapy, Jordan.

1. Introduction

Toxoplasma gondii is a single-cell apicomplexan parasite which infects mammals and birds worldwide causing toxoplasmosis (Robert-Gangneux and Darde, 2012; Fallahi *et al.*, 2018). Infection with *T. gondii* starts upon the ingestion of the infective stage (sporulated oocysts) with contaminated foods or drinks, often shed with the feces of infected cats. Moreover, toxoplasmosis can be acquired upon the ingestion of raw/undercooked infected mammalian or bird meat containing tissue cysts (Cong *et al.*, 2015).

Toxoplasmosis is highly prevalent in humans worldwide (affecting approximately 30- 50 % of the world population) especially among young adults (Furtado *et al.*, 2011, Flegr *et al.*, 2014). The seroprevalence of toxoplasmosis appears to vary from region to region depending on the exposure to the infective stage (the oocysts) (Furtado *et al.*, 2011). While it can reach up to 47 % in France and 14 % in the United States of America, the highest prevalence of toxoplasmosis was reported to occur in South America where its seropositivity can rise up to more than 80 % (Furtado *et al.*, 2011). In the Middle East and other developing countries, there are no accurate statistics for the prevalence of toxoplasmosis. In Jordan, toxoplasmosis IgG antibodies were detected in 66.5 % in a study performed on undergraduate university females (Obaidat *et al.*, 2015). Furthermore, upon examining the prevalence of toxoplasmosis among Jordanian pregnant women, it was found that it reached up to 66.9%, therefore, detecting *T. gondii* antibodies is recommended especially in women who are reported to have multiple miscarriages/abortions (Jumaian, 2005).

In several previous studies, *T. gondii* was reported as the primary infectious agent of congenital infection. However, recent studies confirmed the clinical importance of *T. gondii* and the importance of the infection reactivation among immunocompromised patients (Robert-Gangneux and Darde, 2012). In immunocompetent individuals toxoplasmosis is usually asymptomatic in 90 % of the cases, while the remaining symptomatic cases exhibit flu-like symptoms, ocular disease or cervical lymphadenopathy (Montoya and Liesenfeld, 2004). Toxoplasmosis is severe and could be fatal for some immunocompromised patients, such as those with acquired immune deficiency syndrome (AIDS) (Fallahi *et al.*, 2018).

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Several previous studies suggested that *T. gondii* is an important opportunistic pathogen in immunocompromised patients. A recent review conducted a global meta-analysis and assessed the prevalence of *T. gondii* infection in immunocompromised individuals upon observing the electronic databases for *T. gondii* infection in HIV/AIDS patients, cancer patients, and transplant recipients in a total of seventy-two eligible studies. In the same review, the estimated pooled prevalence of *T. gondii* infection in cancer patients and the control was 26.0 and 12.1% respectively (Wang *et al*., 2017).

When *T. gondii* affects the host, it is usually transmitted through the gastrointestinal (GI) tract to the body in the tachyzoite form, which in turn will invade various parts of the body (mainly the brain, the muscles and the heart) (Jumaian, 2005). In healthy individuals, *T. gondii* enters a latent/ dormancy phase providing a lifelong immunity against this parasite, but the multiplication of bradyzoite may cause destruction for the tissues forming lesions (Flegr *et al*., 2014). However, if the immunity of the individual is weakened by a disease (HIV, cancer, etc.), or by taking certain medications such as corticosteroids, chemotherapy, radiotherapy, *T. gondii* inactive bradyzoites can be reactivated again and transformed into a tachyzoite stage where they can start an acute infection by attacking the surrounding tissue (Fallahi *et al.*, 2018).

In the present study, the main aim was to investigate the seropositivity for *T. gondii* antibodies (IgG and IgM) in cancer patients. The interest in these patients can be attributed to the fact that cancer patients are considered to be immunocompromised due to the nature of their immunosuppressive therapy and as a result, they are expected to be at a higher risk for acquiring toxoplasmosis (new infection or reactivation from previous infection) compared to controls.

2. Materials and Methods

2.1. Study Design and Recruited Patients

This is a cross-sectional study conducted in the hospitals of the Royal Medical Services from November, 2016 until April, 2017 in order to investigate the presence of anti-*T. gondii* antibodies in cancer patients, and to compare them with healthy normal individuals. The ethical approval to conduct the present study was taken from the Hashemite University Institutional Review Board. Also, each participant signed a consent form individually before blood collection. The study recruited two-hundred cancer patients and ninety healthy individuals as a control. Demographic data were collected from patients and controls using a structured questionnaire where the clinical information for each patient was retrieved from the medical report of each patient.

2.2. Serological Assay (ELISA) for the Detection of Anti-Toxoplasma IgG and IgM Antibodies:

Three to five ml of venous blood were collected from each patient and control individually in plain tubes under sterile conditions. The sera were separated, and preserved at -20 °C until being examined. The serological technique, ELISA, was used for the determination of anti-*T. gondii* IgG and IgM antibodies in the serum of the recruited cancer patients and controls. The ELISA kit was provided by a commercial manufacturer (NovaTec®, Germany). The manufacturer instructions were followed for the serum samples and controls and the researchers employed the manufacturer cut-off value of 10 IU/mL of anti-*Toxoplasma* antibody (IgG and IgM) to differentiate between positive (latent, pre-existing or current infection) and negative values.

2.3. Statistical Analysis

Statistical analyses were performed using SPSS statistical computer software for WINDOWS (version 20, IBM Inc., USA). Statistical software was used for the calculation of the seroprevalence of toxoplasmosis among cancer patients and its correlation with variables such as age and sex. The Chi-square test was used for group comparisons (cancer versus normal). Statistical significance was set at 5 %, and all the associations that showed a $P < 0.05$ were considered significant.

3. Results

Using ELISA method, IgG antibodies to *T. gondii* were found in seventy-nine cancer patients (39.5 %) and in eleven of the healthy controls (12.2 %). However, IgM antibodies were found in five cancer patients (2.5 %) and in only one healthy control (1.11 %) (Table 1). The difference in the IgG seropositivity between the patient and the control group is statistically significant ($P < 0.05$). In contrast, the difference in IgM seropositivity between cancer patient and the control group was not statistically significant. The seropositive rates of anti-*T. gondii* IgG were higher in lung, uterine, thyroid and lymph node cancers (50 %), and the lowest rates were observed in stomach and gastric cancer (16.7 %). No anti-*T. gondii* IgG and IgM were detected in the blood of patients with esophageal, bronchial, chest wall mass and pelvic cancers. The seropositive rates of anti-*T. gondii* IgM was observed only in patients with colon and bone cancer, and their ratios were as follow: 17.7 % and 66.7 % respectively. The results were not statistically significant ($P < 0.05$) (Table 2). The seropositivity of anti-*T. gondii* IgG and IgM among the different age groups of cancer patients are shown in Table 3. The highest seropositivity was observed in the age group of 41-50 years in both patients and controls (45.2 % and 66.7 % respectively), (Table 3). The seropositivity in males and females were 42.3 % and 38 %, in cancer patients and 14.3 % and 11.6 % in the controls, respectively. There was no statistical difference in the seropositivity of anti-*T. gondii* IgG and IgM among males and females (Table 3).

Table 1. Seroprevalence of *Toxoplasma gondii* IgG and IgM antibodies in cancer patients and control groups

Antibody	Cancer patients (n = 200)		Healthy controls (n = 90)		*P*- value
	No.	%	No.	%	
Anti-*T.gondii* IgG	79	39.5	11	12.2	< 0.05*
Anti-*T.gondii* IgM	5	2.5	1	1.1	0.44

* Results of Chi-square tests by *P* value of <0.05 as significant difference

Table 2. The seropositivity rates of anti-*T. gondii* IgG and IgM in the cancer patient according to the type of cancer

Cancer type	No. patients	IgG-positive		IgM-positive	
		No.	%	No.	%
Breast cancer	65	28	43.1	0	0.0
Leukemia	20	8	40.0	0	0.0
Lymphoma	18	5	27.8	0	0.0
Colon cancer	17	7	41.2	3	17.7
Myelodyplastic syndrome (MDS)	10	4	40.0	0	0.0
Lung cancer	8	4	50.0	0	0.0
Uterine cancer	8	4	50.0	0	0.0
Stomach and gastric cancer	6	1	16.7	0	0.0
Rectal cancer	6	2	33.0	0	0.0
Myeloma	4	1	25.0	0	0.0
Liver cancer	3	1	…	0	…
Bone cancer	3	1	…	2	…
Esophageal cancer	3	0	…	0	…
Pancreatic cancer	3	1	…	0	…
Bronchial cancer	2	0	…	0	…
Thyroid cancer	2	1	…	0	…
Chest wall mass cancer	2	0	…	0	…
Pelvic cancer	2	0	…	0	…
Lymph node cancer	2	1	…	0	…
Others	16	10	62.5	0	0.0

Seropositivity rates were not shown when the total number of patients was less than 3.

Table 3. *Toxoplasma gondii* IgG seropositivity in cancer patients and controls according to age and gender.

	Cancer patients (n =200)		*Healthy controls (n=90)*	
Factors	Total No. (Positive %)	*P*- value	Total No. (Positive %)	*P*- value
Age (years)				
<20	6 (16.7)		6 (0)	
21-30	11 (18.2)		35 (11.4)	
31-40	28 (39.3)	0.35	27 (3.70)	*<0.05
41-50	42 (45.2)		3 (66.7)	
>51	113 (40.7)		19 (21.1)	
Total	200 (39.5)		90 (12.22)	
Gender				
Male	71 (42.3)		21 (14.3)	
Female	129 (38)	0.55	69 (11.6)	0.39
Total	200 (39.5)		90 (12.2)	

* Results of Chi-square tests by *P* value of <0.05 as significant difference

4. Discussion

During the present study, the results indicated that cancer patients have higher *T. gondii* seroprevalence compared to controls (39.5 % in the cancer patients and 12.2 % in controls) which indicate that cancer patients are at a higher risk for acquiring toxoplasmosis than normal individuals as reported by a previous study (Wang *et al.*, 2017). The seroprevalence observed in this study was close to the *T. gondii* seroprevalence observed in a case–control study of nine-hundred cancer patients and nine-hundred controls in China where the seroprevalence was (35.56 %) and (17.44 %) in cancer patients and the controls, respectively (Cong *et al.*, 2015). Furthermore, the prevalence in the present study (39.5 %) is higher than that observed in two similar studies on cancer patients in Saudi Arabia and Egypt. Among 137 and 150 cancer patients a seroprevalence of 30.6 % and 20 % was observed in Saudi Arabia and Egypt, respectively (Wassef *et al.*, 2016, Imam *et al.*, 2017). Contrary to that, a previous seroprevalence study on cancer patients in Turkey reported a much higher seroprevalence reaching up to (63.0 %) (Yazar *et al.*, 2004).

In the present study, 2.5 % of the cancer patients and 1.1 % of the controls were positive for *Toxoplasma* IgM antibodies which indicates a recent / current infection. On the other hand, 2.0 % of the cancer patients and 1.1 % of the control had both IgG and IgM antibodies which indicate an old and a current infection. In the present study, it is very important to notice that the cancer patients with positive IgM are presented with new *T. gondii* infection which might be acquired from the environment or during multiple blood transfusions. In such compromised patients, the disease might progress directly to active toxoplasmosis, so immediate treatment is required. A recent case report described a disseminated toxoplasmosis after stem cell transplantation in a leukemia toxoplasmosis seronegative patient (Osthoff *et al.*, 2013). The prevalence of toxoplasmosis is observed to be high in general among immunocompromised groups. One study investigated 394 Chinese patients at intensive care units (ICU), and found that 18.78 % of them were positive for anti-*T. gondii* IgG antibodies demonstrating a latent infection (Zhang *et al.*, 2015).

Consistent with many previous studies, the present study observed that the seroprevalence of toxoplasmosis increased with age in both cancer patients and the controls where the highest seropositivity was observed with age >51 (Zhang *et al.*, 2015, Imam *et al.*, 2017). The rise in seroprevalence with age might be a reflection of the increase in the exposure and greater chance to *T. gondii* infection, as the human being gets older (Imam *et al.*, 2017). Also, multiple minor infections might at first produce low antibody levels which are not detectable and which may later reach higher detectable levels as the individual ages (Robert-Gangneux and Darde, 2012). In the current study, there was no statistically significant difference in toxoplasmosis seroprevalence between the recruited males and females in both cancer patients and the controls. However, in many other previous studies, the prevalence of toxoplasmosis was said to be affected by the sex of the individual; some indicated higher seroprevalence in males compared to females, while others showed the opposite indicating that the seroprevalence is

not affected by the sex of the individual (Konishi *et al*., 2000, Imam *et al*., 2017). It is worth mentioning that the seropositivity is not always an accurate evidence of a latent infection in cancer patients since they might receive multiple blood transfusions (from seropositive donors), that could lead to passively transferred antibodies (Imam *et al.*, 2017, Fallahi *et al.*, 2018).

A pervious study in Egypt indicated that toxoplasmosis was higher in patients having solid organ tumors (24 %) than in patients with hematological malignancies (12 %) (P = 0.06) (Wassef *et al*., 2016). The present study showed that the seropositivity was higher in lung, uterine, thyroid and lymph node cancers (50 %) compared to other types of cancer; however, actually no convincing reason can be seen to explain the difference.

In conclusion, the results of the present study showed that cancer patient are at a higher risk for acquiring toxoplasmosis than normal individuals, and these immunocompromised cancer patients should be routinely screened for this parasite in order to put them on early therapy, and prevent severe dissemination. Physicians should start a treatment protocol if anti-*Toxoplasma* IgM antibodies were detected in the cancer patient's serum to avoid any complications of toxoplasmosis.

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