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Study the Possible Link Between Toxoplasmosis and Different Kinds of Cancer in Iraq

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Abstract

Introduction: *Toxoplasma gondii* is an obligate intracellular protozoan parasite which infects a wide range of warm-blooded animals and humans. Cancer remains a leading cause of death worldwide, responsible for approximately 13% of global deaths. Worldwide, the prevalence of human infection with *T. gondii* has been increasing. However, little is known about the epidemiology of *T. gondii* infection in different cancer patient groups in Iraq. Thus, the objective of the present study was to determine the prevalence of anti-*T. gondii* antibodies in patients with four different types of cancer (breast, rectum, thyroid, and leukemia).

Methods: Blood samples were collected from 300 patients with cancer and 150 apparently healthy controls and the sera were tested for the presence of anti-*T. gondii* antibodies (IgG and IgM) using enzyme linked immunosorbent assay (ELISA).

Results: The prevalence of anti-*T. gondii* IgG in cancer patients (49.0%) was significantly higher ($P < 0.001$) than that in controls (19.3%). The highest *T. gondii* IgG positivity rate was detected in breast cancer group (56.6%) followed by rectal cancer group (54.0%), thyroid cancer group (44.6%) and leukemia cancer group (36.0%). It is interesting to note that three male patients were infected with breast cancer and only one (33.3%) was seropositive with IgG. In rectum, thyroid gland, and leukemia cancer groups, the anti-*T. gondii* IgG seropositivity was higher in males than in female patients but the differences were significant ($P < 0.05$) only in rectum and leukemia groups. The highest seropositivity rates were detected in breast, rectal and leukemia cancer patients aged 51 to 60 years.

Conclusions: In conclusion, Toxoplasmosis is significantly more prevalent in cancer patients than in apparently healthy volunteers and accordingly we recommend routine screening of all cancer infected patients for IgG anti-*Toxoplasma gondii* antibodies and treat the seropositive ones.

INTRODUCTION

Toxoplasma gondii is an obligate apicomplexan intracellular protozoan parasite and considered the most common global parasite which infects a wide range of warm-blooded animals and is the etiological agent of one of the most common parasitic infections in humans [1]. About one-third of the world human population has antibodies to *T. gondii* [2].

Although *Toxoplasma gondii* was discovered in 1908, its full life cycle was not discovered until 1970 when it was found that it is a coccidian parasite of cats with all non-feline warm blooded animals (including humans) as intermediate hosts [3]. The life cycle includes three forms: oocysts, tachyzoites, and bradyzoites. The oocysts are only produced in the final hosts (members of the family Felidae) and passed in feces. When oocysts are ingested by the intermediate hosts, they develop into tachyzoites (rapidly multiplying form) which divide rapidly in cells, causing tissue destruction and spreading the infection. Finally, the tachyzoites found in the muscle tissues and the central nervous system transform to tissue cysts, or bradyzoites. Ingestion of cysts in contaminated meat is also a source of infection, as bradyzoites transform back into tachyzoites upon entering a new suitable intermediate host.

It is well known that the common sources of infection are oocysts in cat faeces contaminating drinking water or unwashed vegetables, undercooked meat containing tissue cysts, organ transplants from infected donors containing tissue cysts, and from infected women to her fetus via placenta. However and recently, Flegr et al. [4] hypothesized that toxoplasmosis can be transmitted sexually depending the facts that *Toxoplasma* tachyzoites are present in the seminal fluid and tissues of the testes of various animals including humans, and that in some species, infection of females by artificial insemination with semen from infected males has been observed. Another fact that up to two thirds of *Toxoplasma* infections in pregnant women cannot be explained by the known risk factors and the prevalence of toxoplasmosis in women in child-bearing

age covaries with the incidence of sexually transmitted diseases in particular countries.

The Seropositivity level varies widely in different regions of the globe, measuring between 30% and 60% in most countries [5]. Pappas et al. [6] evaluated the global status of *T. gondii* seroprevalence and its correlations with risk factors, environmental and socioeconomic parameters via reviewing the literature published during the last decade in women who were pregnant or of childbearing age and found that a total of 99 studies were eligible. The authors reported that the seropositivity levels ranged from 0.8% to 77.5% and that foci of high prevalence exist in Latin America, parts of Eastern/Central Europe, the Middle East, parts of south-east Asia and Africa. The regional seroprevalence changes according to social and cultural habits, geographic factors, climate, religious and socioeconomic practices and transmission route, and the prevalence is higher in warm and humid areas [7-9].

In Iraq and its neighbor countries, the seropositivity rates vary considerably. In Iran, the seroprevalance of toxoplasmosis varied between 29.4% and 63.9% in pregnant and childbearing aged women as shown in various studies [10-14]. In Turkey, the seropositivity rates varied from 30.1% to 60.4% in pregnant women [15] while in Kuwait, the rate was 45.7% in pregnant women [16]. In Jordan, the seropositivity rate was 47.1% [17] while in Iraq the rate was 44.7-49.2% in pregnant women [18, 19] and 36.1% in non-pregnant women [20].

The prevalence of human infection with *T. gondii* has been increasing worldwide due to the increasing number of cats. Although the majority of immunocompetent men and non-pregnant women develop asymptomatic lifelong latent infection after being infected by *Toxoplasma gondii* [21], in immunocompromised individuals, as seen in human neoplastic infections, there is an increased risk of reactivation of latent infection in various organs [2]. Moreover, in immunocompromised patients, the infection most often involves the nervous system, with diffuse encephalopathy, meningoencephalitis or cerebral mass lesions [22]. It is well known that *T. gondii* is the most opportunistic protozoan parasite in immunocompromised individuals, especially patients with AIDS, and its involvement in many clinical conditions such as lymphoreticular neoplasias, solid organ transplants has been well documented [23]. When toxoplasmosis acquired during pregnancy, it causes spontaneous abortion, still birth, intrauterine growth retardation, preterm deliveries and fetal abnormalities like hydrocephalus with mental retardation, ocular damage and fetal death which is apparent only in 3rd trimester or after birth [2, 24].

Cancer constitutes an enormous burden on society affecting both developing and developed countries and based on GLOBOCAN estimates [25], about 14.1 million new cancer cases and 8.2 million deaths occurred in 2012 worldwide. The occurrence of cancer is increasing because of the growth and aging of the population, as well as an increasing prevalence of established risk factors such as smoking, overweight, and physical inactivity [26]. In Iraq, a recent study [27] showed that the proportion of breast cancer in females was 33.81%. In comparison with the other Arabic countries, the rate reported by Al-Hashimi and Wang [27] was very similar to that reported in Lebanon but lower than that observed in several Arab countries such as, Kuwait, Jordan and Bahrain. In contrast, the rate was higher than that observed in other Arab countries such as Saudi Arabia, UAE, Qatar, Oman and in non-Arab neighboring countries such as Turkey and Iran [26].

However, little is known about the epidemiology of *T. gondii* infection in patients who are immunocompromised by having neoplastic disease and/or immunosuppressive therapy and has received relatively little attention in Iraq. Thus, the objective of the present study was to study the possible relationship between *T. gondii* infection and cancer via determining the seropositivity rate of anti *T. gondii* antibodies (IgG and IgM) in Iraqi patients with four different cancers using enzyme linked immunosorbent assay (ELISA).

METHODS

Subjects

Three hundred (214 women and 86 men) cancer patients who presented to Al-Amal Hospital, Baghdad, Iraq in 2015 and 2016 and 150 healthy control volunteers were included in the present study. The age of the cancer patient groups was 19 to78 with the mean age of 52.6 ± 14.8 and 27 to 64 with mean age of 51.5 ± 13.6 in the volunteer (control) group.

Collection Of Blood Samples

Approximately 5 ml of venous blood samples were drawn from participants who gave their consent to participate in this study. Blood was taken from all the cancer patients and control group under sterile conditions by a registered nurse, the sera were separated and stored at -20˚C until further testing.

Serological Technique

In this study, enzyme linked immnunosorbent assay (ELISA) was used for determination of anti-*T. gondii* IgG and IgM antibodies in the sera of both cancer patients and the healthy control volunteers according to the manufacturer's instructions. The ELISA kit was provided by a commercial manufacturer (Acon, U.S.A). Positive and negative serum controls were included in every plate and the serology test was done double blinded. Samples from cancer patients and control group were randomly mixed, and the person performing the test did not know the source of samples in advance.

The procedure was done according to the instructions of the manufacturer. Briefly, one well of the ELISA kit plate was left to serve as blank. In the remaining wells, 100 µL of standards, positive, negative controls and samples were added. The plate was incubated for 60 minutes at room temperature and then the contents of the wells were drained and washed 5 times with wash solution. Except for the blank well, 100 µL of the enzyme conjugate was added into each well and then incubated for 30 minutes at room temperature and then the contents of the wells were drained and washed using the wash solution. To all wells, including the blank one, 100 µL of TMB (tetra methyl blue) substrate solution was added and incubated in the dark for 15 minutes at room temperature. In order to stop the reaction, 100 µL of stop solution was added and the absorbance was read within 30 minutes at a wavelength of 450-620 nm using a plate reader. For interpretation of quantitative results in this study, IgG antibody levels greater than 14.5 IU/mL were considered positive while IgM antibody levels of more than 1.1 UL/mL were considered positive.

Statistical Analysis

All data were analyzed using Statistical Programme for the Social Sciences (SPSS) version 22 (IBM SPSS, Chicago, Illinois, USA). Chi square was used to determine relationships and differences between variables as appropriate. The results in comparisons between groups were considered significantly different if $P \leq 0.05$.

RESULTS

The seropositivity of the antibodies of *Toxoplasma gondii* identified by ELISA in cancer patients and apparently healthy volunteers (control group) are shown in Table 1. The results of the present study showed that the overall percentage of seropositivity for *T. gondii* IgG antibodies in cancer patients was significantly higher ($P < 0.05$-0.001) than that among the healthy control subjects. Anti-*T. gondii* IgG antibodies were found in the sera of 147 out of 300 (49.0%) cancer patients and in the sera of 29 out of 150 (19.3%) healthy volunteers. In contrast, anti-*T. gondii* IgM antibodies were found in the sera of only one cancer patients (0.33%) while none of the healthy volunteers were seropositive for IgM.

Table 1: Total Number of Seropositives for *Toxoplasma gondii* Antibodies (IgG and IgM) Among Cancer Patients and the Apparently Healthy Volunteers (Control Group)

Type of Antibody	Cancer Patients			Healthy Controls		
	No. Tested	No. Positive	Percentage	No. Tested	No. Positive	Percentage
Anti-T. gondii IgG	300	147	49.0	150	29	19.3
Anti-T. gondii IgM	300	1	0.33	150	0	0.0

ELISA was used for determination of anti-*T. gondii* IgG and anti-*T. gondii* IgM antibodies.

It can be seen from Table 2 that the highest *T. gondii* IgG positivity rate was detected in breast cancer group (56.6%) followed by rectal cancer group (54.0%), thyroid cancer group (44.6%) and leukemia cancer group (36.0%). The statistical analysis showed that all cancer groups had significantly higher ($P < 0.05$-0.01) seropositivity rate in comparison with the control group. In addition, the breast cancer and rectal cancer groups showed significantly higher ($P < 0.05$) seropositivity rate than other caner groups and no significant difference was detected between the two groups.

Table 2: The Seroprevalence Rate (as a Percentage) of *Toxoplasma gondii* IgG and IgM Antibodies in Cancer Patients and in the Apparently Healthy Volunteers (Control Group)

Cancer Cite	Number of Subjects	IgG Positive, No. (%)	IgM Positive, No. (%)
Breast	106	60 (56.6) [a]	1 (0.9)
Thyroid Gland	94	42 (44.6) [a]	0 (0.0)
Rectum	50	27 (54.0) [a]	0 (0.0)
Leukemia	50	18 (36.0) [b]	0 (0.0)
Total	300	147 (49.0)	1 (0.33)
Control Group	150	29 (19.3) [c]	0 (0.0)

ELISA was used for determination of anti-*T. gondii* IgG and anti-*T. gondii* IgM antibodies.
Values in the same column sharing the same superscript letters are not significantly different from each other.

Regarding the effect of gender on the seropositivity of anti-*T. gondii* IgG antibodies, it is interesting to note that three male patients were infected with breast cancer and only one (33.3%) was seropositive with anti-*T. gondii* IgG (Table 3). In rectum, thyroid gland, and leukemia cancer groups, the anti-*T. gondii* IgG seropositivity was higher in males (62.1, 50.0, and 42.9%, respectively) than in female patients (42.9, 42.6, and 27.3%, respectively) and the differences were significant ($P < 0.05$) in both rectum and leukemia groups. In control group, the percentage of seropositivity was marginally higher in female (21.0%) than in male (16.0%) apparently healthy subjects but the difference was not significant (Table 3).

Table 3: Gender Wise Distribution of Anti-*Toxoplasma gondii* IgG Seropositivity in Cancer Patients and Healthy Volunteers (Control Group)

Cancer Group	Number Tested		Number Positive		Positivity, %	
	Males	Females	Males	Females	Males	Females
Breast	3	103	1	59	33.3 [a]	57.3 [b]
Rectum	29	21	18	9	62.1 [a]	42.9 [b]
Thyroid gland	26	68	13	29	50.0 [a]	42.6 [b]
Leukemia	28	22	12	6	42.9 [a]	27.3 [b]
Control	50	100	9	21	16.0 [a]	21.0 [a]

Values in the same row sharing the same superscript letters are not significantly different from each other.

It can be seen from Table 4 that the highest seropositivity rates were detected in breast, rectal and leukemia cancer patients aged 51 to 60 years while in thyroid cancer, the highest positivity rate was in patients aged 41 to 50 years. Similarly, the highest seropositivity rate was detected in control subjects aged 41 to 50 years.

Table 4: Age Distribution of Seropositive Cases for *Toxoplasma gondii* IgG Antibodies Among Cancer Patients and Apparently Healthy Volunteers (Control Group)

Age Group, Y	Breast Cancer		Thyroid		Rectum		Leukemia		Controls	
	NT	NP (%)	NT	NP (%)	NT	NP (%)	NT	NP (%)	NT	NP (%)
20-30	3	1 (33.3)	9	4 (44.4)	0	0 (0.0)	4	1(25.0)	44	4 (9.1)
31-40	8	1 (12.5)	13	6 (46.2)	3	1 (33.3)	7	2(28.6)	42	10 (23.8)
41-50	30	18 (60)	22	13 (59.1)	9	3 (33.3)	6	1 (16.7)	43	11(25.6)
51-60	40	25 (62.5)	29	9 (31.0)	17	11 (64.7)	17	7 (41.2)	17	3 (17.6)
61-70	19	14 (73.7)	17	8 (47.1)	17	10 (58.8)	15	6 (40.0)	4	1 (25.0)
> 71	6	1 (16.7)	4	2 (50.0)	4	2(50.0)	1	1 (100.0)	0	0 (0.0)
Total	106	60 (56.6)	94	42 (44.7)	50	27(54.0)	50	18 (36.0)	150	29 (19.3)

Abbreviations: NT, number tested; NP, number positive.

Table 5 shows residency wise distribution of the anti-*T. gondii* IgG seropositivity in both cancer patients and control subjects. In all cancer groups, the seropositivity was significantly higher ($P < 0.05$) in rural patients than in urban patients (Table 5). In contrast, the seropositivity was significantly higher ($P < 0.05$) in the apparently healthy subjects living in urban areas than their counterparts living in rural areas.

Table 5: Residency Wise Distribution of Anti-*Toxoplasma gondii* IgG Seropositivity in Cancer Patients and Apparently Healthy Volunteers (Control Group)

Group	Number Tested		Number Positive		Positivity, %	
	Rural	Urban	Rural	Urban	Rural	Urban
Breast	24	82	17	43	70.8 [a]	52.4 [b]
Rectum	14	36	8	19	57.1 [a]	52.8 [b]
Thyroid gland	20	74	10	10	50.0 [a]	13.5 [b]
Leukemia	13	37	12	6	92.3 [a]	16.2 [b]
Control	20	130	2	27	10.0 [a]	20.8 [b]

Values in the same row sharing the same superscript letters are not significantly different from each other.

DISCUSSION

The principal finding of the current study was that the prevalence of anti-*T. gondii* IgG in cancer patients (49.0%) was significantly higher than that in apparently healthy volunteers (control group; 19.3%) which may indicate the presence of an association between *T. gondii* infection and some kinds of cancer, having probability of cancer patients being more prone to *T. gondii* infection, or *T. gondii* infection contributing to development of some kinds of cancer, but this needs further studies in order to prove that. Similarly, Yazar et al. [28] investigated the seropositivity rate of toxoplasmosis in Turkish patients with neoplasia to determine levels of anti-*T. gondii* IgG and IgM antibodies and their results revealed higher percentages of positivity for *T. gondii* IgG antibodies in patients with neoplasia (52.9%) compared with the controls (20%) with a statistically significant difference. The authors concluded that these findings may be due to the fact that patients with neoplasia are immunocompromised, which increases their susceptibility to infection with toxoplasmosis. Yuan et al. [29] conducted study to determine *T. gondii* antibodies in 267 Chinese cancer patients (116 men and 151 women) by using ELISA and they found higher positivity rates of *T. gondii* IgG in cancer patients than the control individuals. Among the cancer patients, the authors reported that the positivity rates of *T. gondii* IgG in nasopharyngeal carcinoma and rectal cancer groups were significantly higher than the other cancer groups, while the differences in IgM positivity rates were not significant. The authors concluded that there is a possible link between *T. gondii* infection and some kinds of cancer, especially nasopharyngeal carcinoma and rectal cancer. Indirectly and without giving a good evidence, Yuan et al. [29] suggested that cancer paves the way for toxoplasmosis based on the fact that patients with malignant neoplasia are immunocompromised, which increases their susceptibility to *T. gondii*. In contrast, the opposite could be true as Thomas et al. [30] explored associations between *T. gondii* and brain cancers in human populations and they predicted that *T. gondii* could increase the risk of brain cancer because it is a long-lived parasite that encysts in the brain, where it

provokes inflammation and inhibits apoptosis. The results of their study showed that infection with *T. gondii* was associated with a 1.8-fold increase in the risk of brain cancers. The authors concluded that *T. gondii* should be investigated further as a possible oncogenic pathogen of humans. Another study conducted by Vittecoq et al. [31] showed that mortality rates due to brain cancer in France were correlated positively with the local seroprevalence of *T. gondii*, particularly in the people who are 55 years of age or older. Recently, Cong et al. [32] collected blood from 900 Chinese cancer patients and 900 controls to detect anti-*T. gondii* antibodies by ELISA and reported that the prevalence of anti-*T. gondii* IgG in cancer patients (35.6%) was significantly higher than that in controls (17.4%). Their results also showed that the highest *T. gondii* seroprevalence was detected in lung cancer patients (60.94%), followed by cervical cancer patients (50%), brain cancer patients (42.31%) and endometrial cancer patients (41.67%). The authors concluded that *T. gondii* infection is a severe problem in cancer patients and improved measures should be conducted to prevent and control the infection in cancer patients.

Although these studies may provide strong evidences suggesting that *T. gondii* is associated with brain and other cancers, it is unclear how the infection causes cancer in humans. Accordingly, Thirugnanam et al. [33] present a hypothesis that *T. gondii* infection may have the ability to modulate the host microRNAs and could potentially promote the development of brain cancer. Their hypothesis predicts that *Toxoplasma*-modified microRNAs may play a critical role in initiation and progression of brain carcinogenesis, though the outcome of the infection possibly depends on the mode of infection, parasitic strain, type of host cell and microRNA expression patterns of host cell and parasite proteins. The authors concluded that further research on the specific microRNA pathways affected by *Toxoplasma* in various brain cells would open new avenues in the diagnosis and treatment of brain cancers caused by *Toxoplasma* infection.

On the other hand, a recent pioneer study conducted by Sanders et al. [34] published unexpected and very interesting finding in that attenuated *T. gondii* stimulates immunity to pancreatic cancer by manipulation of myeloid cell populations. The authors put a hypothesis that treatment of established aggressive disseminated pancreatic cancer with the immunotherapeutic attenuated *T. gondii* vaccine strain CPS would trigger tumor-associated myeloid cells to generate therapeutic antitumor immune responses and their results validate their hypothesis in that CPS treatment significantly decreased tumor-associated macrophages and markedly increased dendritic cell infiltration of the pancreatic tumor microenvironment. In addition, CPS treatment increased CD4(+) and CD8(+) T-cell infiltration into the tumor microenvironment, activated tumor-resident T cells, and increased IFNγ production by T-cell populations. Moreover, CD8(+) T cells isolated from CPS-treated tumor-bearing mice produced IFNγ after re-exposure to pancreatic tumor antigen, suggesting this immunotherapeutic treatment stimulates tumor cell antigen-specific CD8(+) T-cell responses. The authors concluded that CPS treatment provided a significant therapeutic benefit in pancreatic tumor-bearing mice via targeting tumor-associated myeloid cells as a mechanism to stimulate more effective immunity to pancreatic cancer. Although the results of this pioneer study are very interesting, unfortunately cannot be compared with the results of the current study and those of Yuan et al. [29], Thomas et al. [30], Vittecoq et al. [31], and those of Cong et al. [32] because the strain used in Sanders et al. [34] study was experimentally immunotherapeutic attenuated *Toxoplasma gondii* vaccine while those in the present study and other studies were not attenuated and supposedly pathogenic strains.

In contrast, *T. gondii* has the ability to manipulate host cell signaling pathways and processes by interfering with the gene expression profiles of the invaded cells [35] which in turn respond by initiating apoptotic response which reduces survival and proliferation of the parasites and makes the parasites susceptible to immune attack. However, some studies have shown that *T. gondii* has established several strategies to neutralize the extrinsic and intrinsic cellular suicide programs of the infected cells [35, 36]. Moreover, intracellular infection with *T. gondii* turns host cells resistant to multiple inducers of apoptosis, including Fas-dependent and Fas-independent cytotoxic T-lymphocytes (CTL)-mediated cytotoxicity, interleukin (IL)-2 deprivation, gamma irradiation, ultraviolet (UV) irradiation, and calcium ionophorebeauvericin [37].

The results of the above mentioned studies including the results of the current study simply point to a correlation between *T. gondii* and different types of cancers, but do not indicate that the parasite could promote the development of cancer because the opposite may be true based on the fact that brain and other types of cancers can deteriorate the immune system, which might make the infection with toxoplasmosis more likely. Accordingly, the authors of the present study think that both ways are possible, meaning the cancer paves the way for toxoplasmosis and toxoplasmosis promotes the development of cancer depending on which one comes first because both diseases weakened the immune system and both are associated with defects in cell-mediated immunity [38]. In addition to the weakened immune systems, other factors could affect the risk of both cancer and toxoplasmosis such as cell phone use (which has been linked to brain cancer) [31]. Significantly higher positivity rates of *Toxoplasma gondii* IgG were detected in cancer patients than the in the control group which may suggest a possible link between toxoplasmosis and cancer. We propose that when individuals are previously infected with chronic toxoplasmosis and then get the infection with any sort of cancer, the chance of reactivation of the latent infection will be high and at the same time the opportunity for the cancer to be more aggressive will also be high. In contrast, when individuals are having any sort of cancer and then get the infection with *T. gondii*, toxoplasmosis will be aggressive and the opportunity for the acute infection to last longer (instead of changing into chronic one due to the active immune system) will be ideal because of the weakened immune system due to cancer itself or its immunosuppressive therapy. As long as no treatment is available for cancer, the treatment of toxoplasmosis will be greatly helpful for the cancer patients to decrease the pressure on the immune system when both diseases are concurrently present. Accordingly, cancer patients should be monitored for *T. gondii* routinely and should be kept away from infection sources of toxoplasmosis.

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CONFLICTS OF INTEREST

There is no conflict of interest.

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