

Brief Original Article

The relationship between toxoplasmosis and different types of human tumors

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Abstract

Introduction: *Toxoplasma gondii* is an intracellular protozoan that may disrupt the traditional cell barriers against cancer, allowing the accumulation of oncogenic mutations over time. Our research aimed to explore the relationship between *T. gondii* infection and tumor development.

Methodology: The anti-*T. gondii* IgG and IgM antibodies were tested for 156 patients with tumors (51 with breast cancer, 20 with hepatoma, 20 with larynx carcinoma, 20 with squamous cell carcinoma of bone, 16 with lymphoma, 13 with brain tumor, 4 with bladder cancer and 12 with benign uterine tumor) and 90 healthy controls by using electrochemiluminescence immunoassay. Tissue specimens were collected from *T. gondii* seropositive cases for histological and immunohistochemistry (IHC) examinations. **Results:** The seroprevalence of human toxoplasmosis in the Sharkia Governorate, Egypt is significantly correlated with various types of tumors: breast cancer in 49 subjects (96.1%), and squamous cell carcinoma of bone in 16 subjects (80%). It was also present in nine cases of brain tumors. Anti-*Toxoplasma* IgG was detected in seven cases of liver tumors and one-quarter of bladder cancer. The anti-*Toxoplasma* IgM was present in three patients with benign uterine tumors, one patient with a bone tumor and two patients with breast cancer. *Toxoplasma* cysts were detected in immunostained brain sections.

Conclusion: The correlation between *T. gondii* infection and tumors was established by this study indicating a significant emerging role of human toxoplasmosis in the etiology or existence of particular types of tumors.

Key words: human toxoplasmosis; malignancy; humoral immunity.

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Introduction

Cancer is the leading cause of death in developed countries. Many studies demonstrate the association between the parasitic infection and human cancers [1-2].

Toxoplasmosis is present in every country with (10% - 90%) seropositivity [3]. *Toxoplasma gondii* is an intracellular protozoan parasite that alters the surrounding provision of the infected host then invades vital organs. Human toxoplasmosis has frequently been mild or self-limiting in immunocompetent patients but serious in immunodeficient ones [4]. Tissue cysts, which are controlled by the cellular immune system, represent its sustainability inside the host [5]. This parasite can persist in active form for a longer period within immunologically advantaged sites such as the

brain and the eye [6]. *Toxoplasma* is an important pathogen associated with the occurrence of gliomas and meningioma through infection-induced modulation of the mouse macrophage proteome [2]. This interactive connection between *T. gondii* and the host macrophages has been confirmed [7]. Serologic evidences for the presence of anti-*T. gondii* IgG and IgM have been correlated with many cancer diseases [8]. The regional mortality rates in China were correlated with the seroprevalence of human toxoplasmosis, suggesting the need for further investigation concerning this parasitic infection as a possible oncogenic pathogen in humans [6-9].

Karin *et al.* stated that long-term defensive host responses resulting from persistent infections might promote malignancy by provoking inflammation that

increases the mutation rates [10]. The intracellular pathogens may disrupt the traditional cell barriers against cancer, allowing the accumulation of oncogenic mutations [11]. Much evidence suggests that toxoplasmosis may be associated with human malignancies [12].

This study aimed to explore the relationship between *T. gondii* infection and tumor development. The seroprevalence of anti-*T. gondii* IgG and IgM were investigated in Sharkia Governorate, Egypt by the use of electrochemiluminescence.

Methodology

Population study

This case-control study was performed on 156 tumor patients who were diagnosed in the early stage before taking chemotherapy, and were referred to Zagazig University Hospital, Al-Ahrar Hospital and the Faqous Cancer Center during 2016. Cases with suppressed immune systems or advanced cancer were excluded. The admission rate of cancer patients is about 13 cases per month. The study included 90 healthy control volunteers. The patients and the control subjects were classified according to their ages as <30, 30-50 and 50-70. The cases included in this study were breast cancer, primary intraosseous squamous cell carcinoma, squamous cell carcinoma of the mandibular bone, brain tumors {glioblastoma & astrocytoma}, laryngeal carcinoma, bladder cancer, liver tumors, lymphoma and a benign tumor of the uterus.

Ethics

This study was approved by the Ethics Committee of the Zagazig University Faculty of Medicine, Egypt and written consent was obtained from all the participants.

Collection of Blood Samples

Approximately 5 mL of venous blood was drawn from each patient. The blood samples were labeled and cooled at 4°C during transport to the laboratory. The blood samples were then centrifuged to obtain sera in 1.5 mL centrifuge tubes. The serum samples were stored at -80°C until used.

Determination of anti- *Toxoplasma* IgG and IgM Antibodies

The serum samples were examined using the electrochemiluminescence immunoassay ECLIA (Cobas E411 immunoassay analyzers, Mannheim, Germany) for qualitatively determine the avidity of the

anti-*Toxoplasma gondii* IgG and IgM antibodies in the samples.

Histopathology and Immunohistochemistry

Tissue specimens from the liver, breast, brains, laryngeal lymph nodes, urinary bladder and uterine tumors were collected from the anti- *T. gondii* seropositive cases and fixed in neutral-buffered 10% formalin and prepared for histological examinations. The presence of *T. gondii* tissue cysts was investigated by immunohistochemistry (IHC) using stained sections of the brains and livers of the seropositive cases. Thin tissue sections (4 µm) were deparaffinized and hydrated, and the endogenous peroxidase was blocked with a 3% hydrogen peroxide solution. The sections were incubated for 30 min with primary rabbit anti-*T. gondii* antibodies (Neomarkers, Fremont, USA), diluted to 1:200. After rinsing with phosphate buffered saline (PBS), the tissues were incubated with biotin-conjugated secondary antibodies (Lab Vision Corporation, Fremont, USA). A streptavidin– biotin system was used for a one-hour incubation at room temperature. Diaminobenzidine (DAKO, Burlingame, USA) was used as the chromogen, and the slides were counterstained with Mayer's hematoxylin

Reference measurement

A lower detection limit and the maximum of the master curve is defined by 0.13- 650 IU/mL. Values below the lower detection limit were reported as < 0.13 IU/mL and that above measuring range were reported as > 650 IU/mL.

Statistical analysis

Data were tabulated and statistically analyzed using SPSS program version 11. Fisher's exact test was used to compare qualitative variables. The Student's t-test was used in comparisons between quantitative variables. A p-value equal to or < 0.05 was considered significant.

Results

Participants

A total of 156 tumor patients in the age range of 30 - 70 years with median age of 50 years were included in the study. Of 156 patients, females accounted for 42.30%, and males constituted about 57.7% of studied cases. Ninety healthy controls were collected at the same age of cases in which females represented 44.4%, and males represented 55.6% within significant difference ($p > 0.05$).

Table 1. Seropositivity of *T. gondii* IgG and IgM using electrochemiluminescence among the studied groups.

Variable	Cases (n = 156)		Control (n = 90)		p	
	n	%	n	%		
<i>T. gondii</i>	Negative	63	40.4	58	64.4	< 0.001
	Positive IgG	87	55.8	27	30	
	Positive IgM	6	3.8	5	5.6	

P^, Chi square test; Highly significant (p < 0.001).

Table 2. Seropositivity of *T. gondii* IgG and IgM by electrochem-iluminescence in relation to the gender of the studied groups.

Group	<i>T. gondii</i>	Male		Female		p
		n	%	n	%	
Cases	Negative	(n = 90)		(n = 66)		< 0.001
	Positive IgG	63	70	0	0	
	Positive IgM	27	30	60	90.9	
Control	Negative	(n = 50)		(n = 40)		< 0.001
	Positive IgG	44	88	14	35	
	Positive IgM	6	12	21	52.5	

P^, Chi square test; Highly significant (p < 0.001).

A total of 156 tumor patients constituted 51 breast cancer, 20 liver tumor, 20 larynx carcinoma, 20 squamous cell carcinoma in bone, 16 lymphomas, 13 brain tumor {glioblastoma & astrocytoma}, 4 bladder cancer and 12 benign uterine tumor.

Anti- *T. gondii* IgG antibodies were detected in sera of 87 (55.8%), and IgM in 6 {3.8%} out of the 156 studied cases (Table 1) with a highly significant difference (p < 0.001).

Concerning the gender, the frequencies of anti-*Toxoplasma* IgG was 27 (30%) in male cases and 60 (90.9%) of females with a highly significant difference (Table 2). IgM antibodies were only detected in 6 (9.1%) of women with a highly significant difference (p < 0.001).

Anti-*Toxoplasma* IgG were detected in 49 (96.1%) breast cancer, 16 (80%) squamous cell carcinoma of bone, 9 (69.2%) brain tumor {glioblastoma and astrocytoma}, 7 (35%) liver tumor, 1 (25%) bladder cancer, 2 (16.7%) benign tumor in uterus and 1 (5%) in

laryngeal carcinoma. The positive rate of *Toxoplasma* IgM was detected in 3 (25%) benign tumors of uterus, 1 (5%) squamous cell carcinoma in bone and 2 (3.9%) breast cancer with highly significant difference (p < 0.001) (Table 3).

Histopathologic examination revealed intracranial calcifications in association with an astrocytoma in *T. gondii* seropositive patients (Figure 1A). A total of 18.75% (3/16) of tumor tissue evaluated (brain and liver tumors) were positive for *T. gondii* antibody by IHC (Figure 1B).

Discussion

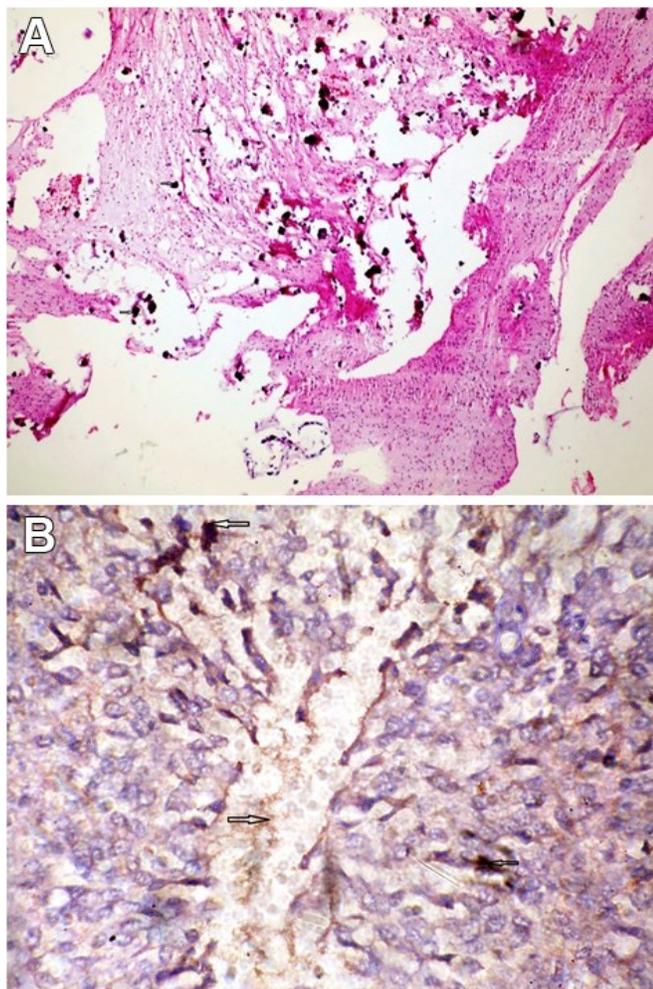
Establishing a link between human *T. gondii* infection and tumors could open the door for probable revenue of reducing cancer threat. This study aims to explore the relationship between *T. gondii* infection and tumor development. We investigated the seroprevalence of *T. gondii* among different tumor

Table 3. Seropositivity of *T. gondii* IgG and IgM by electrochem-iluminescence among different types of tumors in studied cases.

Variable	No	IgG		IgM		p
		n	%	n	%	
Breast cancer	51	49	96.1	2	3.9	< 0.001
Squamous cell carcinoma in bone	20	16	80	1	5	
Brain tumor	13	9	69.2	0	0	
Laryngeal carcinoma	20	1	5	0	0	
Benign tumor in uterus	12	2	16.7	3	25	
Bladder cancer	4	1	25	0	0	
Liver tumor	20	7	35	0	0	
Lymphoma	16	2	12.5	0	0	

P^, Chi square test; Highly significant (p < 0.001).

Figure1. A) Hand E stained section of astrocytoma in patient with toxoplasmosis revealing numerous calcification (Hand E $\times 200$) (arrow). B) Immunostained section of *T. gondii*-infected human brain (astrocytoma) with numerous cysts containing bradyzoites (IHC $\times 400$) (arrow).



patients diagnosed in Sharkia Governorate / Egypt during 2016.

In our study, out of 156 studied cases, anti-*T. gondii* IgG antibodies were detectable in sera of 87 (55.8%), and IgM was detected in 6 (3.8%) with a highly significant difference ($p < 0.001$), as seen in Table 1. Our results are relatively in compliance with reports from couple of countries. In a study in Turkey anti-*T. gondii* IgG antibodies were detected in 68 (63.0%) patients with a neoplasia and 21 (19.4%) healthy volunteers by ELISA with a statistically significant difference [13]. Similarly, in a study in Latin American countries in which *T. gondii* seroprevalence was over 60% [3]. Higher seroprevalence (68.7%) was reported in Nepalese cancer patients [14]. This difference may be as a result of different geographical factors, eating habits and livestock farming practices.

The frequencies of anti-*Toxoplasma* IgG antibodies were detected in 27 (30%) of males and 60 (90.9%) of females with the highly significant difference ($p < 0.001$). Whereas IgM antibodies were detected only in 6 (9.1%) of women with a highly significant difference ($p < 0.001$). These results suggested higher prevalence than that of Wang *et al.* who reported 8.96% prevalence of antibodies to *T. gondii* in male cancer patients and 7.45% in females. These findings could be due to the geographical variation and genetic susceptibility [15]. As the functions of the immune system are disturbed in patients with aneoplasm [16], toxoplasmosis has been described in association with some malignancies [13].

In our study, the highest positivity of IgG was detected in breast cancer (96.1%) followed by squamous cell carcinoma in the bone (80%), brain tumor (69.2%), liver tumor (35%), bladder cancer (25%), benign tumor in uterus (16.7%), then Laryngocarcinoma (5%) (Table 3). Moreover, the positive rate of IgM antibodies was in benign tumors of uterus (25%), breast cancer (3.9%) and the squamous cell carcinoma in bone (5%) with highly significant difference ($p < 0.001$). Our results of breast cancer were complied with others who registered 93.7% positivity for *T. gondii* antibodies in women with breast cancer [17]. Yazar *et al.* reported (63.0%) anti-*T. gondii* IgG in patients with neoplasm and (19.4%) in healthy volunteers with statistically significant difference ($p < 0.05$) by using ELISA, (6.5%) IgM antibodies in patients with neoplasm and (0.9%) in healthy volunteers with statistically significant differences [13]. ELISA was used for detection of anti-*T. gondii* IgG and IgM antibodies in tumor patients and approved the association between *T. gondii* infection and nasopharyngeal carcinoma [6]. Jung *et al.* revealed 18.3% *T. gondii* seropositivity among brain tumor patients in Korea ($p < 0.05$) using ELISA [18]. Conversely, Wang *et al.* reported a positive rate of 8.38% in cancer patients for toxoplasmosis [15]. This difference may be due to the nature of their study conducted on immune-suppressed patients and genetic susceptibility in China. On the other hand, cps, avirulent, strain of *T. gondii*, which invades immunosuppressive CD11c β antigen-presenting cells in the ovarian carcinoma was used as therapy for natural immunity of highly immunosuppressive microenvironment of the most solid tumor [19]. This may be as a result of using a different *Toxoplasma* strain besides, their research on ovarian cancer that was not included in our research.

Conclusion

Results of the present research suggest that the seroprevalence of human toxoplasmosis in the province of Sharkia Governorate Egypt is significantly correlated with the presence of various types of tumors: breast cancer, squamous cell carcinoma in bone, brain tumor {glioblastoma and astrocytoma}, liver tumor, bladder cancer, and a benign tumor in the uterus. The association between *T. gondii* infection and tumors was established in our study demonstrating a significant emerging role of toxoplasmosis in the etiology or existence of particular types of tumor providing an approach for the management of the observed tumors.

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