

High *Toxoplasma gondii* Seropositivity among Brain Tumor Patients in Korea

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Abstract: *Toxoplasma gondii* is an intracellular protozoan that can modulate the environment of the infected host. An unfavorable environment modulated by *T. gondii* in the brain includes tumor microenvironment. Literature has suggested that *T. gondii* infection is associated with development of brain tumors. However, in Korea, epidemiological data regarding this correlation have been scarce. In this study, in order to investigate the relationship between *T. gondii* infection and brain tumor development, we investigated the seroprevalence of *T. gondii* among 93 confirmed brain tumor patients (various histological types, including meningioma and astrocytoma) in Korea using ELISA. The results revealed that *T. gondii* seropositivity among brain tumor patients (18.3%) was significantly ($P < 0.05$) higher compared with that of healthy controls (8.6%). The seropositivity of brain tumor patients showed a significant age-tendency, i.e., higher in younger age group, compared with age-matched healthy controls ($P < 0.05$). In conclusion, this study supports the close relationship between *T. gondii* infection and incidence of brain tumors.

Key words: *Toxoplasma gondii*, seroprevalence, brain tumor, ELISA

More than 14.1 million common cancer cases are estimated around the world in 2012, and this number is anticipated to increase to 24.0 million by 2035 [1]. About 256,000 central nervous system tumors (1.8% of all tumors) are diagnosed each year worldwide [1]. Previous studies have found that risk factors for brain tumors include diverse chemical products, family history, and ionizing radiation from therapeutic and diagnostic devices in the head [2]. However, the exact causes of these malignancies are yet unclear. Approximately 20% of various malignancies worldwide are due to infectious agents, including viruses, bacteria, and parasites [3,4]. Infectious agents can interfere with the host cell genetic machinery, such as DNA repair and cell cycle, and can lead to chronic inflammation and immune system impairments [5].

The infectious agents associated with human cancers are most commonly viral pathogens, including human papilloma virus and hepatitis B and C viruses [6]. However, there are only a few studies on the association between parasites and human cancers. It has been suggested that several parasite species, namely,

Paragonimus westermani, *Plasmodium* sp., *Opisthorchis viverrini*, *Clonorchis sinensis*, *Schistosoma haematobium*, and *Hymenolepis nana* are related to development of various types of human cancers [7-8]. Many other species of parasites may also have potential roles in development of human cancers.

Toxoplasma gondii is an intracellular protozoan that can modulate the microenvironment of the infected host [9]. *T. gondii* can invade vital organs, including the central nervous system (CNS); however, its infection in humans is usually mild and asymptomatic in immunocompetent individuals [9]. In immunocompromised patients, *T. gondii* infection may cause severe diseases in the brain, including fatal meningitis and encephalitis [9]. In addition, a possible correlation was suggested between *T. gondii* infection and brain tumor development [10]. This suggestion has been supported by other authors [11-18]. The mechanisms of the brain tumor induction by *T. gondii* need to be further studied. In this study, in order to estimate the possible relationship between *T. gondii* infection and brain tumor development, we investigated the seroprevalence of *T. gondii* among brain tumor patients diagnosed in Korea.

We used sera of 93 patients (44 men and 49 women) diagnosed with various types of brain tumors supplied by the Biobank of Chonnam National University Hwasun Hospital (Hwasun, Jeollanam-do Province, Korea), a member of the Korea Biobank Network. As the control group, sera of 93 ran-

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domly selected healthy volunteers (45 men and 48 women) who visited the Korea Association of Health Promotion for health check-up were included for the assay. The ages of the brain tumor patients were 18-82 years (52.9 ± 14.8 years) and those of the healthy controls were 10-86 years (51.9 ± 12.2 years). The sera and blood were stored at -80°C until analyzed. This study protocol was approved by the Institutional Review Board of Seoul National University Hospital, Seoul, Korea (IRB no. E-1507-065-687). The purpose and procedures of this study were explained to all participants, and a written informed consent was obtained from each of them.

The process of preparation for *Toxoplasma* lysate antigens (TLA) [19] and the procedure of ELISA [14] followed previous studies with slight modifications. Briefly, 96-well microtiter plates (Costar, Cambridge, Massachusetts, USA) coated with TLA were incubated at 4°C overnight. After washing, each well was reacted with the test serum samples (1:100) at 37°C for 1 hr, and horseradish peroxidase-conjugated goat anti-human

IgG (1:10,000; Bethyl Laboratories, Montgomery, Texas, USA) was applied at 37°C for 1 hr. After several washes, freshly prepared o-phenylenediamine dihydrochloride (Sigma-Aldrich, St. Louis, Illinois, USA) was added, and the reaction was stopped by adding 8 N H_2SO_4 . IgG antibody titers were determined at the optical density of 490 nm. To analyze the risk factors for toxoplasmosis, the chi-square test was applied. $P < 0.05$ was considered statistically significant. Pearson's chi-square and Fisher's exact tests were used to investigate associations among qualitative categorical variables using SPSS (SPSS Inc., Chicago, Illinois, USA). All tests were 2-sided, and the level of significant difference was defined as $P < 0.05$.

The IgG seropositive rate for *T. gondii*, as analyzed by ELISA, was 18.3% (17/93) among patients with variable types of brain tumors (Table 1). The seropositivity among brain tumor patients was significantly ($P < 0.05$) higher than the one among healthy persons, 8.6% (6/93 sera). In particular, patients with meningioma (41.7%; $P < 0.05$), metastatic carcino-

Table 1. Seropositivity of *T. gondii* by ELISA among brain tumor patients in Korea

Types of brain tumors	Brain tumor patients		Healthy controls	
	No. tested	No. positive (%)	No. tested	No. positive (%)
Meningioma	12	5 (41.7) ^a		
Metastatic carcinoma	10	3 (30.0) ^a		
Astrocytoma (including pilocytic/oligo)	14	3 (21.4)		
Glioblastoma	31	5 (16.1)		
Ganglioglioma and others ^b	26	1 (3.8)		
Total	93	17 (18.3)	93	8 (8.6)

^aSignificantly higher than healthy controls ($P < 0.05$).

^bInclude ependymoma, pituitary adenoma, diffuse large b-cell lymphoma, oligodendroglioma, brain parenchymal tissue with minimal pathology, chordoma, craniopharyngioma, epidermoid cyst, hemangioblastoma, neuroendocrine type, sinonasal adenocarcinoma.

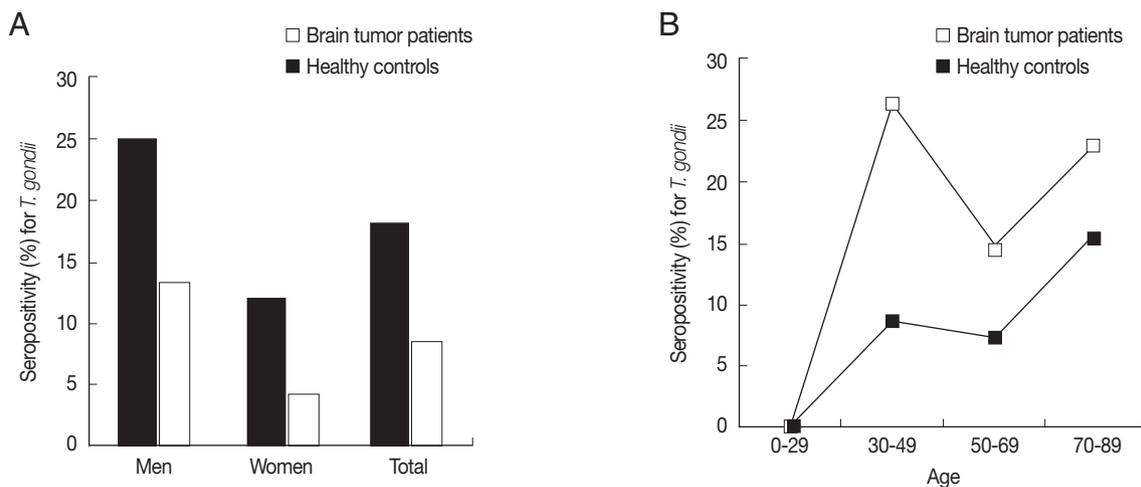


Fig. 1. Gender (A) and age (B)-associated seroprevalence of *T. gondii* infection among brain tumor patients in Korea compared with healthy controls.

ma (30.0%; $P < 0.05$), astrocytoma (21.4%; $P > 0.05$), and glioblastoma (16.1%; $P > 0.05$) showed remarkably higher seropositivity for *T. gondii* than healthy controls (Table 1). The seropositivity was significantly ($P < 0.05$) higher in men than in women both in the brain tumor patients and healthy controls (Fig. 1A). The age-seropositivity curve in healthy controls generally showed a steady increasing pattern according to increase of the age (Fig. 1B), whereas in brain tumor patients the younger age group, for example, 30-49 years, revealed a significantly ($P < 0.05$) higher seropositivity compared with other age groups (Fig. 1B).

Previous studies have shown that meningioma and astrocytoma were positively linked to high serum *T. gondii* IgG antibody levels [10,13]. Similarly, our study showed higher prevalence of *Toxoplasma* specific IgG in patients with meningioma (41.7%; $P < 0.05$) and astrocytoma (21.4%; $P > 0.05$) (Table 1). Furthermore, in healthy controls, the seropositivity of *T. gondii* was increased with age [19,20]. However, in our study, the seropositivity in brain tumor patients showed a different tendency; a significantly higher rate was observed in younger aged patients (30-49 years) with brain tumors, compared with age-matched healthy controls. These results support strongly the close relationship between *Toxoplasma* infection and brain tumor incidence.

Since the 1960s, quite a number of studies [10-18] reported correlations between *T. gondii* infection and brain tumor incidence (Table 2). Various types of brain and multiple organ tumors, including glioma, acoustic neuroma, meningioma, Hodgkin's lymphoma, multiple myeloma, and leukemia, have

been reported to be positively associated with chronic *T. gondii* infection (Table 2). In this study, we also observed a high prevalence of *T. gondii* infection in sera of various brain tumor patients in Korea. Especially, our study supports the previous finding that IgG seropositivity to *T. gondii* is a risk factor for meningioma [13]. These findings provide a strong need for a further study to establish the precise correlation between meningioma incidence and *T. gondii* infection. However, results in this study have limitation to precisely explain the relationship between *T. gondii* infection and brain tumor.

To evade host immune responses, *T. gondii* parasites transform themselves into tissue cysts, and they are parasitic on various tissues, including the brain, heart, and skeletal muscle for lifetime of the host modulating the host immune responses [21]. Furthermore, they can also modulate the cell cycle and apoptosis of the host cells for their proliferation [17,22]. Such behavioral pathogenesis of *T. gondii* infection may be linked to the related hallmarks of tumor development. The unfavorable environment modulated by *T. gondii* may be similar to appropriate precancerous conditions. Thus, we can presume that *T. gondii* infection should help to develop brain tumors. Hosts with chronic *T. gondii* infections are also vulnerable to the attack by other pathogens such as viral pathogens. If such viral pathogens are associated with brain tumor development, it can be another oncogenic effect of *T. gondii* and may synergistically act as a carcinogen. In this respect, it is noteworthy that *Plasmodium falciparum* and Epstein-Barr virus contributed synergistically to the formation of Burkitt's lymphoma [23].

Despite the studies on the association of *T. gondii* and brain

Table 2. Summary of previous studies on the association of *T. gondii* infection with tumor incidence

Year	Country	Tumor type	Methods	Results	Reference
1963-1964	USA	Glioma, Acoustic neuroma, Meningioma, Others	Sabin-Feldman dye-test	Tumor patients (n=126): 56.3% Healthy controls (n=126): 41.3%	Schuman et al. [11]
1979-2007	France	Brain tumor	Database	Brain tumor mortality rates increase with <i>T. gondii</i> seroprevalence in France.	Vittecoq et al. [12]
1987-1990	Australia	Glioma, Meningioma	ELISA (IgG)	Tumor patients (n=53): 47.0% Healthy controls (n=348): 31.0%	Ryan et al. [13]
2000-2002	Turkey	Hodgkin's lymphoma, Multiple myeloma, Leukemia, Others	ELISA (IgG)	Cancer patients (n=108): 63.0% Healthy controls (n=108): 19.4%	Yazar et al. [14]
2006	China	Nasopharyngeal carcinoma, Rectal cancer, Others	ELISA (IgG)	Cancer patients (n=267): 24.0% Healthy controls (n=148): 6.1%	Yuan et al. [15]
2008	Korea	Malignant neoplasms	LAT, ELISA (IgG)	Malignant neoplasms: 19.0% (16 cases/84 <i>T. gondii</i> positive cases)	Shin et al. [16]
2008	37 countries	Brain tumor	Database	Infection with <i>T. gondii</i> was associated with a 1.8-fold increase in the risk of brain tumors.	Thomas et al. [17]
2012-2014	China	Brain tumor, Lung cancer, Cervical cancer, Others	ELISA (IgG)	Tumor patients (n=900): 35.6% Healthy controls (n=900): 17.4%	Cong et al. [18]

tumors, we cannot conclude whether chronic *T. gondii* infection, with seropositivity, is involved in causing these brain tumors, or reversely brain tumor patients are at risk of recrudescence and dissemination of *T. gondii* to undergo a chronic infection [14]. Researches are also needed to determine whether *T. gondii* itself acts as a carcinogen or takes the role of a creator of a precancerous environment to develop into brain tumors.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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