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 westernmani, 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-
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 ($52.9 \pm 14.8$ years) and those of the healthy controls were 10-86 years ($51.9 \pm 12.2$ 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 $8 \text{ N H}_2\text{SO}_4$. 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>
<thead>
<tr>
<th>Types of brain tumors</th>
<th>Brain tumor patients</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>No. positive (%)</td>
</tr>
<tr>
<td>Meningioma</td>
<td>12</td>
<td>5 (41.7)$^a$</td>
</tr>
<tr>
<td>Metastatic carcinoma</td>
<td>10</td>
<td>3 (30.0)$^a$</td>
</tr>
<tr>
<td>Astrocytoma (including pilocytic/oligo)</td>
<td>14</td>
<td>3 (21.4)</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>31</td>
<td>5 (16.1)</td>
</tr>
<tr>
<td>Ganglioglioma and others$^b$</td>
<td>26</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>17 (18.3)</td>
</tr>
</tbody>
</table>

$^a$Significantly higher than healthy controls ($P<0.05$).
$^b$Include 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](image_url). Gender (A) and age (B)-associated seroprevalence of *T. gondii* infection among brain tumor patients in Korea compared with healthy controls.
Table 2. Summary of previous studies on the association of T. gondii infection with tumor incidence

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

**ACKNOWLEDGMENTS**

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**CONFLICT OF INTEREST**

We have no conflict of interest related to this work.

**REFERENCES**