The prevalence of anti-Toxoplasma gondii antibodies in stutterers is higher than in the control group

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Abstract: The purpose of this study was to investigate the possible relationship between Toxoplasma gondii (Nicolle et Manceaux, 1908) and stuttering. We investigated the seropositivity rate for anti-T. gondii IgG and antibodies by enzyme-linked immunosorbent assay (ELISA) in stuttering children to ascertain a possible relationship between T. gondii infection and stuttering. We selected 65 stuttering children and 65 control children (non-stutterers) to investigate the seropositivity rate of anti-T. gondii antibodies by ELISA. Cranial magnetic resonance imaging (MRI) and scalp electroencephalography (EEG) were also performed in stuttering children. The seropositivity rate of anti-T. gondii IgG antibodies among stuttering children (28%) was significantly higher than in control group (5%; p = 0.001). No abnormality was detected in cranial MRI’s of stuttering children and their EEG recordings were also normal. There was no significant difference in seropositivity rate regarding age, genders and residence area. The association between seroprevalence of infection with T. gondii and stuttering may be due to hyperdopaminergic state in brains of patients who are T. gondii-seropositive. Thus, there might be a causal relationship between toxoplasmosis and stuttering.

Keywords: toxoplasmosis, stuttering, enzyme-linked immunosorbent assay (ELISA), electroencephalography (EEG), magnetic resonance imaging (MRI)

Toxoplasma gondii (Nicolle et Manceaux, 1908) is a protistan parasite that infects up to a third of the world’s population (Montaya and Liesenfeld 2004). It has clinical manifestations ranging from a completely asymptomatic infection to multiorgan involvement (Montaya and Liesenfeld 2004). Human beings can be infected through eating raw or undercooked meat of infected animals containing tissue cysts, or through accidental ingestion of oocysts excreted by infected cats. Other routes of infection include maternal-fetal transmission that can cause congenital toxoplasmosis (CT), blood transusions, solid organ or hematopoietic cell transplantation and laboratory accidents (Kapperud et al. 1996, Montaya and Liesenfeld 2004).

Prenatal, perinatal or childhood exposure to T. gondii have important roles in the development of behavioural and neuropsychiatric disorders (Fabiani et al. 2013). Most children with congenital toxoplasmosis are developmentally normal but up to 4% die or have permanent neurological damage or bilateral visual impairment during the first years of life (Gras et al. 2005, Salt et al. 2005).

Toxoplasma gondii has a strong tropism for central nervous system (CNS). Its dormant stages, tissue cysts, are mostly localised in cerebral hemispheres, basal ganglia, cerebellum and brain stem. Toxoplasma gondii affects CNS both directly and indirectly. The parasite infects neurons and glial cells, mainly astrocytes (Halonen et al. 1996, Cotter et al. 2001), and produces tissue cysts. Especially in immunocompromised patients, it causes serious neuropathological involvement resulting in acute necrotising encephalitis and glial nodules (Conejero-Goldberg et al. 2003). Mild hemiparesis is the most common focal finding. Headache, confusion, lethargy, brain stem and cerebellar disorders, and seizures may also be seen.

These clinical features are similar to those of primary central nervous system lymphoma (PCNSL), which presents with confusion, lethargy, memory loss, hemiparesis, speech and language disorders, seizures, and cranial nerve palsies, in descending order of frequency (Porter and Sande 1992). Stuttering is a speech disorder defined by frequent prolongations, repetitions or blocks of spoken sounds and/or...
syrllables (Andrews et al. 1983). Stuttering is a prevalent developmental disorder of early childhood. It can appear suddenly after a period of normal speech and language development. It involves genetics and is believed to be a problem with neural processing of speech and possibly involving the basal ganglia (Alm 2004, Smits-Bandstra and De Nil 2007, Onslow and O’Brian 2013). However, despite thorough research etiology of stuttering has not been clearly defined yet (Bloodstein and Bernstein Ratner 2008).

To investigate a possible association between T. gondii infection and stuttering, we tested the hypothesis that a positive antibody titre (IgG) reflects chronic infection and the presence of tissue cysts within the CNS or other body tissues.

**MATERIALS AND METHODS**

**Participants**

Our study included 65 stuttering children and 65 children as control of the age of 4–14 years who were admitted to Medical School Child and Adolescent Psychiatry Department of the Gaziantep University between January 2013 and January 2015. The study group included children who were diagnosed as stuttering according to DSM (The Diagnostic and Statistical Manual of Mental Disorders) IV diagnostic criteria (American Psychiatric Association 2000). The control group included children who did not have any speech disorder. All participants lived in city centre of Gaziantep province.

Anti-Toxoplasma gondii IgG antibodies were assessed in sera of both groups of children. Children with a diagnosis of stuttering were examined by a pediatric neurologist after variables such as age, sex, duration of stuttering and family history were recorded. To evaluate possible neurological impairments in stuttering children in whom anti-T. gondii IgG antibodies were detected, cranial magnetic resonance imaging (MRI) and scalp electroencephalography (EEG) were performed. The study was approved by the University of Gaziantep Human Research Ethics Committee.

**Serum collection and laboratory assay**

Latent toxoplasmosis is a lifelong illness in humans and thus the presence of anti-T. gondii IgG antibodies is considered as a reliable indicator of viable bradyzoites in tissue cysts that persist in infected individuals (Montoya and Liesenfeld 2004). Therefore, blood samples were obtained from the patients by venipuncture to measure the concentration of anti-T. gondii IgG antibodies. Sera were separated from whole blood and were stored at -80°C until tested. The levels of class specific immunoglobulin G (IgG) antibodies against purified T. gondii antigens in the serum samples were measured using enzyme-linked immunosorbent assay (ELISA), which was performed according to manufacturer’s instructions (Cobas Core, Roche, Langen, Germany).

**Statistical analysis**

Statistical analysis was carried out using SPSS software (version 13.0) and Kolmogorov-Smirnov test was used to determine whether the data was distributed normally. Testing of the association between duration of stuttering and toxoplasmosis was performed with independent samples t-test and effect of age on this association was tested with ANCOVA where duration of stuttering was taken as dependent variable, toxoplasmosis status as independent variable and age as a covariate. The results were reported as mean ± SD. Categorical variables were compared using the Chi-square test and were expressed as counts and percentages. A value of P < 0.05 was considered statistically significant.

**RESULTS**

Using ELISA method, anti-Toxoplasma gondii IgG antibodies were detected in 18 of 65 (28%) children with diagnosis of stuttering and 3 of 65 (5%) children without a diagnosis of stuttering. When we compared the results, seropositivity rate of T. gondii IgG antibodies were significantly higher in stuttering children (OR = 7.91, CI 95% = 2.20–28.45, p = 0.005) (Table 1). In stuttering children 39 of 65 cases (60%) were males and mean age was 9.8 ± 2.9 years. In control group, 35 of 65 (54%) children were males and their mean age was 9.2 ± 3.2. The prevalence of stuttering was found to be similar in girls and boys (p > 0.05). Mean age of all children was 9.4 ± 3.1 years and there was not any age difference between groups (p > 0.05). Mean duration of stuttering was 4.7 ± 2.8 years and there was not any correlation between duration of stuttering and presence of anti-T. gondii IgG antibodies (p = 0.211).

To test the effect of age on this relation, ANCOVA was performed in which status of infection with T. gondii was taken as independent variable, whereas duration of stuttering was dependent variable and age was covariate. We found that age significantly affected duration of toxoplasmosis (p = 0.000) and after elimination of effect of age on duration of stuttering, T. gondii status still did not affect duration of stuttering (p = 0.418). There was no significant difference in seropositivity rate regarding age, genders and residence area respectively (p = 0.724, p = 0.479, p = 0.323) (Table 2).

Neurological examinations of subjects were within the normal ranges. No abnormality was detected in cranial MRI’s of stuttering children and their EEG recordings were also normal.

**DISCUSSION**

To the best of our knowledge there is no previous study investigating stuttering and seropositivity to Toxoplasma gondii. Seroprevalence in our stuttering group (28%) was higher, whereas in control group lower (5%) than the seroprevalences reported for similar age groups by several other authors. Jones et al. (2001) found 23% seroprevalence of T. gondii in the United States in subjects ≥ 12 years. Ali et al. (2007) found seroprevalence of anti-T. gondii IgG of 10% in primary school children (mean age 9.7 ± 1.6) in

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**Table 1.** Presence of antibodies against Toxoplasma gondii (Nicolle et Manceaux, 1908) in stuttering children and in control groups.

<table>
<thead>
<tr>
<th></th>
<th>IgG (+)</th>
<th>IgG (-)</th>
<th>Total</th>
<th>p-value</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stuttering children</td>
<td>18 (28%)</td>
<td>47 (72%)</td>
<td>65</td>
<td>0.005</td>
<td>7.9 (2.2–28.5)</td>
</tr>
<tr>
<td>Control group</td>
<td>3 (5%)</td>
<td>62 (95%)</td>
<td>65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR – odds ratio; CI – confidence interval.
Iran, Taylor et al. (1997) 12.8% in children 4–18 years old, Sadaruddin et al. (1991) 17.4% in children 13–20 years old, and Chomel et al. (1993) 3.1% in children 13–15 years old. There is no study on prevalence of toxoplasmosis in children at school ages in Turkey.

It has been recently demonstrated that latent infection with T. gondii can alter behavioural parameters of man and rodents (Miman et al. 2010). Several studies have also demonstrated possible association of latent toxoplasmosis with obsessive compulsive disorder, epilepsy, depression and migraine (Halici et al. 2001, Yazar et al. 2003, Koseoglu et al. 2009, Miman et al. 2010, Flegr et al. 2014). It was also reported that RhD-negative subjects with high titres of anti-T. gondii antibodies had a probability of a traffic accident of about 17%, which was more than six times higher than T. gondii-free or RhD-positive subjects (Flegr et al. 2009). Other studies also found the higher risk for traffic accidents in seropositive group (Flegr et al. 2002, Yereli et al. 2006, Kocayebek et al. 2009, Alvarado-Esquível et al. 2012) and nearly significant correlation was found between prevalence of toxoplasmosis and incidence of traffic accidents in 87 countries (Flegr and Dama 2014).

Prevalences of psychiatric disorders have also been found to be higher in T. gondii-seropositive people. Yagmur et al. (2010) found increased suicide attempts in T. gondii-seropositive subjects. Lester (2010) found that national suicide rates among 20 European countries were higher in countries with greater T. gondii prevalence. A study investigating T. gondii prevalence in psychiatric patients found 18.2% seropositivity whereas the rate was 8.9% in controls (Alvarado-Esquível 2006).

Children of mothers who were tested during pregnancy and found to have high anti-T. gondii antibodies have increased prevalence of schizophrenia spectrum disorders. There have been many studies investigating T. gondii-seropositivity in schizophrenic patients. A meta-analysis of 38 studies found that schizophrenia patients had higher T. gondii-seropositivity than healthy controls (odds ratio: 2.71) (Torrey et al. 2012). The most recent meta-analysis showed lower but still significant odds ratio of 1.81 (CI₉₅ = 1.51–2.16) (Sutterland et al. 2015). More recent association of Alzheimer’s disease (AD) with T. gondii-seropositivity has also been investigated. Kusbeci et al. (2011) found increased T. gondii seroprevalence in 34 AD patients compared with healthy groups. This result supports previous findings of association between T. gondii seroprevalence and psychiatric and behavioural disturbances.

Anxiety, in particular, is believed to be among the most common psychological concomitants of stuttering (Menzies et al. 1999), and increasing amount of evidence suggests the presence of social anxiety or social phobia in people who stutter (Iverach et al. 2009).

Based on decrease of personality factors of novelty seeking in T. gondii-infected subjects, Flegr et al. (2003) suggested that increased level of neurotransmitter dopamine plays the key role in toxoplasmosis-associated psychological and psychiatric disturbances. It was observed that tissue cysts of T. gondii have two genes encoding tyrosine hydroxylases, which is the rate limiting step in dopamine synthesis (Gaskell et al. 2009). Recently, extremely high concentrations of dopamine have been found to accumulate in cyst-containing brain cells and in vitro infection was found to cause accumulation of high amounts of dopamine in neural cells (Prandovszky et al. 2011).

Toxoplasma gondii could specifically alter dopamine level in the infected neurons. Dopamine antagonists haloperidol and 1-[2-β(4-fluorophenyl)methoxyethyl]-4-(3-phenylpropyl)piperazine dihydrochloride (GBR 12909) were found to alter behaviours in rats infected with T. gondii (Skallová et al. 2006). A study using a static rod test has shown that T. gondii-infected mice have impaired learning capacity and eight-arm radial maze test but spontaneous activity of these mice was increased in the wheel running test compared to non-infected animals (Hodková et al. 2007).

Excess of dopamine is supposed to play a role in the etiology of stuttering. Dopamine blockers are the most commonly used drugs in stuttering. Also an F-DOPA PET scan in stuttering subjects showed increased uptake in right ventral medial prefrontal cortex, left caudate tail, limbic structures (such as amygdala, left insular cortex and right deep orbital cortex) and auditory complex (Wu et al. 1997). There is also evidence that infection with T. gondii has greatest impact on the hippocampus and amygdala. Vyas et al. (2007) studied bioluminescence imaging of the brain of rats infected with T. gondii. They found higher tissue cyst density in the amygdala region.

The association between seroprevalence of T. gondii and stuttering may be due to hyperdopaminergic state in brains of patients infected with T. gondii and this association may explain the increased prevalence of toxoplasmosis in stuttering children. If confirmed by other studies with samples involving higher numbers of stuttering children, this may lead to new approaches in treatment of stuttering children.

| Table 2. Socio-demographic characteristics of the study populations |
|----------------|----------------|----------------|----------------|
| Stuttering children | Control group | p-value | OR (CI) |
| Age (years)* | (n = 65) | (n = 65) |
| 4-9 | 30 | 28 | 0.724 | 1.11 |
| 10-14 | 35 | 37 | 0.57–2.26 |
| Genders* | | | | |
| Female | 26 | 30 | 0.479 | 0.78 |
| Male | 39 | 35 | 0.39–1.56 |
| Residence* | | | | |
| Urban | 50 | 45 | 0.323 | 1.48 |
| Rural | 15 | 20 | (0.68–3.24) |
| * Chi Square test; OR – odds ratio; CI – confidence interval. Note: Females were coded as ‘0’ and males were coded as ‘1’ to the statistics program. |


Çelik et al.: Anti-\textit{T. gondii} antibody levels in stutterers