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# Possible role of toxoplasmosis in patients with first-episode schizophrenia*

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**Aim:** To determine the possible relationship between toxoplasmosis and patients with first-episode schizophrenia (FES).

**Materials and methods:** Seventy-three subjects with FES (15-54 years old; mean: 23.4 years) and 40 healthy individuals (20-54 years old; mean: 30.3 years) were enrolled in the study. Most of the FES patients (90.4%) and the control individuals (95.0%) were male. Specific IgG and IgM antibodies to *Toxoplasma gondii* were investigated by enzyme-linked immunosorbent assay (ELISA), and the total amount of antibodies was investigated with the Sabin-Feldman dye test (SFDT).

**Results:** Compared to the control individuals, study subjects had significantly more toxoplasmosis-like symptoms and more cats in the household; they were less likely to live in apartments and more likely to live in ground-floor houses. They did consume more uncooked meat, unpasteurized goat's milk, and chicken eggs; had more contact with soil; and were living less often in urban areas and more often in rural areas. Serum samples from 32 (43.8%) and 25 (34.2%) of 73 patients with FES were seropositive for *T. gondii* when tested by ELISA IgG and SFDT, respectively. Out of 40 serum samples from control subjects, 13 (32.5%) and 15 (37.5%) were found positive for *T. gondii* by ELISA IgG and SFDT, respectively. In patients with FES, 17 (68%) out of 25 who were SFDT-positive were also positive by ELISA IgG test, while 15 out of 48 (31.3%) SFDT-negative serums were positive by ELISA IgG. In the control group, 11 out of 15 SFDT-positive serum samples (73.3%) were also positive by ELISA IgG test, while 2 out of 25 SFDT-negative serum samples were positive by ELISA IgG.

**Conclusion:** The present study shows that toxoplasmosis might be associated with first-episode schizophrenia. More studies are needed to prove the association between *T. gondii* infection and patients suffering from schizophrenia.

**Key words:** *Toxoplasma gondii*, toxoplasmosis, schizophrenia, ELISA, Sabin-Feldman dye test

## İlk atak şizofreni olgularında toksoplazmozun olası rolü

**Amaç:** Çalışmanın amacı, ilk atak şizofrenili (FES) hastalar ile toksoplazmozun olası ilişkisini saptamak olmuştur.

**Yöntem ve gereç:** Çalışmaya 73 FES'li (yaşları 15-54 yaş: ortalama 23,4) ve 40 sağlıklı (yaşları 20-54 yaş: ortalama 30,3) birey alınmıştır. Çalışma gruplarından FES'li hastaların (% 90,4) ve kontrol grubunu (% 95) çoğunlukla erkekler oluşturmuştur. *Toxoplasma gondii*'ye spesifik IgG ve IgM antikorları Enzyme Linked Immunosorbent Assay (ELISA) ve total antikorlar Sabin-Feldman dye test (SFDT) ile saptanmıştır.

**Bulgular:** Kontrol grubu ile karşılaştırıldığında, çalışma grubunu oluşturan hasta bireylerin daha fazla toksoplazmoz benzeri semptomlar ve kedi sahibi olmak yönünden istatiksel olarak anlamlı oldukları bulunmuş ve de onların apartman tarzından ziyade toprakla aynı seviyede olan evlerde yaşadıkları saptanmıştır. Çalışma grubu daha fazla pişmemiş ya da çiğ et ile pastörize olmayan keçi sütü ve tavuk yumurtası tüketmekte, daha fazla toprakla temasta bulunmakta ve de şehirden ziyade daha fazla kırsal kesimde yaşamaktaydılar. Serolojik testlere bakıldığında, 73 FES'li hastanın 32'si (% 43,8) ELISA IgG, 25 (% 34,2)'i SFDT ile seropozitif olarak saptanmıştır. Kontrol grubunda, 13'ü (% 32,5) ELISA IgG, 15'i

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(% 37,5) SFDT ile pozitif bulunmuştur. FES'li hastaların analizinde, pozitif 25 SFDT örneğin 17'si (% 68) ELISA IgG ile de pozitif bulunurken 48 SFDT negatif serumun 15'i (% 31,3) ELISA IgG ile seropozitif bulunmuştur. Kontrol grubunda, pozitif 15 SFDT örneğin 11'i (% 73,3) ELISA IgG ile de pozitif bulunurken 2 negatif serum ELISA IgG ile seropozitif bulunmuştur.

**Sonuç:** Çalışma, toksoplazmozun ilk atak şizofreni ile birliktelik gösterebileceğini ortaya koymaktadır. Şizofreniden yakınan hastalar ile *T. gondii* enfeksiyonu birlikteliği hakkında daha fazla çalışmalar yapılmasına gereksinim vardır.

**Anahtar sözcükler:** Toksoplazmoz, şizofreni, *Toxoplasma gondii*, ELISA, Sabin-Feldman dye test

## Introduction

Schizophrenia is a serious neuropsychiatric disease of uncertain etiology. Epidemiological and neuropathological studies have indicated that some cases of schizophrenia may be associated with environmental factors, such as exposure to infectious agents. However, specific infectious agents associated with the development of schizophrenia have not been identified (1). In humans, acute infection with *Toxoplasma gondii* can produce psychotic symptoms similar to those displayed by persons with schizophrenia (2). Consequently, *T. gondii* may be a candidate infectious agent related to schizophrenia.

Toxoplasmosis is caused by *Toxoplasma gondii*, a human pathogen of cosmopolitan distribution. The infection is usually prevented by the host immune response, which is predominately a cellular one; however, once established, it usually persists throughout the entire life of the host (3). The molecular mechanisms of cell invasion by *T. gondii* and those leading to cyst formation are largely unknown, but the preferential location of cysts in the central nervous system might be related to its specific immunological status (4). It was found that the tachyzoite stage of *T. gondii* can infect both astrocytes and neurons. However, higher percentages of astrocytes are infected than neurons, and astrocytes support more replication of *T. gondii* than the latter. In addition, there is evidence that cysts formed in astrocytes may be distinct from neuronal cysts (5), by forming tissue cysts that contain numerous "resting" bradyzoites. Cysts may occur in all tissues, but are most common in muscle and neural tissue, including the brain (6).

In the diagnosis of *T. gondii* infections, the presence of IgG, IgA, and IgM immunoglobulins does not always allow a reliable identification of the exact stage of the infection. The lack of clear, specific clinical signs related to toxoplasmosis makes it difficult to determine the exact time of infection (7).

In Turkey, about 1 out of 3 people become infected with *T. gondii* at some point in their lives, and in parts of continental Europe, up to 80% of the adult population is infected. This zoonotic disease is considered one of the major health problems among parasitic infections. The particular neurotropism of *T. gondii* and its association with congenital brain dysfunction makes it possible to consider a relationship between exposure to this organism and the progression of psychiatric diseases (8). Individuals with schizophrenia may act in ways that increase the probability of becoming infected with *T. gondii* either prior to or after the start of the disorder (8).

The aim of the study was to determine a possible relationship between toxoplasmosis and first-episode schizophrenia.

## Materials and methods

### *Study populations*

The study was carried out at the schizophrenia outpatient unit of the Department of Psychiatry, Gülhane Military Medical Academy, School of Medicine, Ankara, Turkey. The original study group comprised 85 subjects. All subjects who were considered for the present study had been admitted to the schizophrenia outpatient unit consecutively between 2002 and 2006. Twelve of the 85 subjects were excluded from the study because we could not obtain clear information about their history of exposure to infectious agents. In the final sample of 73 subjects, the mean age of the subjects was 23.4 ± 5.9 years (range = 15-54 years). Subjects with a past history of or a concomitant neurological or medical

illness, or persistent or severe substance abuse, were excluded. As the control group, 40 healthy individuals were enrolled in the study. The mean age of the control group was 30.3 ± 8.8 years (range = 20-54 years). The mean age of the control group was significantly higher than that of the study group ($t = 5.02$; $P < 0.001$). The study and control groups consisted mainly of males (90.4% and 95.0%, respectively) (Table 1).

A questionnaire form, prepared by the authors, was used for the assessment of the subjects' basic sociodemographic variables. An additional questionnaire was used for the assessment of the subjects' detailed history of exposure to infectious agents. All subjects were asked about their history of toxoplasmosis-like diseases; the presence of cats in the household and the degree of closeness with these animals; house type; consumption of uncooked or raw meat, unpasteurized goat's milk, and chicken eggs; contact with soil; residence type (urban or rural); and blood transfusion and organ transplant history. Whenever possible, the patient's history was corroborated by medical records and family members.

Schizophrenia was diagnosed using the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) criteria (9). In addition, the diagnosis of first-episode schizophrenia in each participating patient was confirmed by an experienced psychiatrist (Ö.U.) by means of the Structured Clinical Interview for DSM-IV (SCID) (10).

***Ethical aspects***

This study was approved by the Ethical Review Committee of Gülhane Military Medical Academy (GMMA), Turkey. Informed consent was obtained from all subjects.

***Serological tests***

Specific IgG and IgM antibodies to *T. gondii* were detected by ELISA IgG and IgM tests (RADIM, Italy) according to the manufacturer's recommendations.

***Toxoplasma-specific IgG avidity test***

IgG avidity was determined with the *Toxoplasma* IgG avidity test kit (RADIM, Italy). Values of less than 20% indicated low avidity, values between 20% and 30% were considered to have equivocal avidity, and values higher than 30% were considered to have high avidity.

***Sabin-Feldman Dye Test (SFDT)***

The SFDT measures the total amount of antibodies in serum that are capable of complement-mediated killing of *Toxoplasma* tachyzoites. Serum was diluted across a microtiter plate and the end point was defined as the dilution at which 50% of the tachyzoites were dead. The SFDT was considered positive when the titer was above 1/16.

***Statistical analysis***

All statistical analyses were performed using SPSS 10.0 statistical software (SPSS Inc., Chicago, USA). Descriptive statistics were shown in either mean ± standard deviation notation or as frequency tables. Relations among the categorical variables were investigated by chi-square test. P-values less than or equal to 0.05 were considered statistically significant.

## Results

Seven out of 73 FES patients (9.6%) presented a history of toxoplasmosis-like symptoms (fever, myalgia, dizziness, headache, and lympadenopathy), while none had chorioretinitis or visceral disease. Compared to the control individuals, study subjects had significantly more toxoplasmosis-like symptoms and more cats in their household; they were living less often in apartments and more often in ground-floor houses. They did consume more uncooked meat, goat's milk, and chicken eggs; had more contact with soil; and were living less often in urban areas and more often in rural areas (Table 1).

Serum samples from 32 (43.8%) and 25 (34.2%) of 73 patients with FES were seropositive for *T. gondii* when tested by ELISA IgG and SFDT, respectively. Only one subject had low IgG avidity (Table 2). Out of 40 serum samples from the control subjects, 13 (32.5%) and 15 (37.5%) were found positive for *T. gondii* by ELISA IgG and SFDT, respectively. All of these subjects had high IgG avidity. All patients and control subjects were found negative by ELISA IgM.

In patients with FES, 17 (68%) out of 25 who were SFDT-positive were also found positive by ELISA IgG, while 15 out of 48 (31.3%) SFDT-negative serums were positive by ELISA IgG (Table 3). In the control group, 11 out of 15 SFDT-positive serum samples (73.3%) were also positive by ELISA IgG, while 2 out

Table 1. Socio-demographic characteristics of subjects.

| Study subjects | First-episode schizophrenia (n = 73) | Control group (n = 40) | Statistics |
|---|---|---|---|
| Age (years) | 23.4±5.86 | 30.3±8.76 | P < 0.001 |
| Sex (male/female) | 65/8 (90%/10%) | 38/2 (95%/5%) | $\chi^2$ = 0.742, P = 0.488 |
| History of toxoplasma-like disease [a] | 7 (9.6%) | 0 (0%) | $\chi^2$ = 4.08, P = 0.05 |
| Having cat(s) in the household [a] | 15 (28%) | 1 (2.5%) | $\chi^2$ = 7.62, P = 0.006 |
| House type [a] | | | $\chi^2$ = 17.15, P < 0.001 |
| Apartment | 35 (47.9%) | 35 (87.5%) | |
| Ground-floor house without backyard | 7 (10%) | 1 (2.5%) | |
| Ground-floor house with backyard | 29 (41%) | 4 (10%) | |
| Consumption of uncooked or raw meat [a] | 25 (35%) | 3 (7.5%) | $\chi^2$ = 10.7, P = 0.001 |
| Consumption of unpasteurized goat's milk and chicken eggs [a] | 15 (28%) | 0 (0%) | $\chi^2$ = 8.76, P = 0.002 |
| Contact with soil [a] | 40 (56.2%) | 1 (2.5%) | $\chi^2$ = 31.86, P < 0.001 |
| Received a blood transfusion or organ transplant [a] | 1 (1%) | 0 (0%) | - |
| Residence type of community [a] | | | - |
| Urban | 52 (73%) | 40 (100%) | |
| Rural city | 19 (27%) | 0 (0%) | |

[a]No information was available from 4 patients or their guardians.

Table 2. ELISA and SFDT results in subjects with first-episode schizophrenia and healthy individuals.

| | | ELISA IgM | ELISA IgG | ELISA IgG Avidity | Sabin-Feldman Dye Test |
|---|---|---|---|---|---|
| First-episode schizophrenia (n = 73) | Positive | - | 32 | 1[a] | 25 |
| | Negative | 73 | 41 | 72[b] | 48 |
| | Total | 73 | 73 | 73 | 73 |
| Control group (n = 40) | Positive | - | 13 | 0 | 15 |
| | Negative | 40 | 27 | 40[b] | 25 |
| | Total | 40 | 40 | 40 | 40 |

[a]Low avidity, [b]High avidity

of 25 SFDT-negative serum samples were positive by ELISA IgG (Table 3). Only one FES patient showed a low avidity by ELISA IgG. In the study group, 6 out of 25 SFDT-seropositive subjects had a titer of 1/16, 15 had a titer of 1/64, and 4 had a titer of 1/256. In the control group, 6 out of 15 SFDT-seropositive subjects had a titer of 1/16 and 9 had a titer of 1/64.

Six patients with schizophrenia were tested again 6 months after the onset of symptoms for IgM and IgG by ELISA as well as by SFDT. None was positive for IgM, while one patient sero-converted from positive to negative regarding IgG. All patients retained high IgG avidity and 2 who were found positive by SFDT became negative later.

Table 3. Comparison of the SFDT with ELISA in subjects with first-episode schizophrenia and healthy individuals.

| First-episode schizophrenia (n = 73) | Sabin-Feldman Dye Test | ELISA IgG | | ELISA IgM | | ELISA IgG Avidity | |
|---|---|---|---|---|---|---|---|
| | | + | - | + | - | + | - |
| | Positive (n = 25) | 17 | 8 | 1 | 24 | 1 | 24 |
| | Negative (n = 48) | 15 | 33 | - | 48 | - | 48 |
| | Total | 32 | 41 | 1 | 72 | 1 | 72 |
| **Control group (n = 40)** | **Sabin-Feldman Dye Test** | **ELISA IgG** | | **ELISA IgM** | | **ELISA IgG Avidity** | |
| | | + | - | + | - | + | - |
| | Positive (n = 15) | 11 | 4 | - | 15 | - | 15 |
| | Negative (n = 25) | 2 | 23 | - | 25 | - | 25 |
| | Total | 13 | 27 | - | 40 | - | 40 |

## Discussion

In our study, no significant differences were found between FES patients and control individuals regarding seropositivity to *T. gondii.* This might be related to the fact that few seropositive individuals show clinical symptoms and to the high prevalence of toxoplasmosis in healthy individuals. This is in contradiction with other studies that have shown that seropositivity to *T. gondii* infection is significantly higher in schizophrenic patients compared to healthy matched controls (11). Cetinkaya et al. (12) have found that the seropositivity rate of *T. gondii* IgG antibodies in patients with schizophrenia (66%) indicates that chronic *Toxoplasma* infection in these patients is significantly greater than among patients with depressive disorder (24%) or healthy volunteers (22%). Sonmez Tamer et al. (13) detected 40% IgG and 5% IgM seropositivity among schizophrenic patients and found that IgG *T. gondii* antibodies were significantly higher in schizophrenia patients compared with controls. Mortensen et al. (14) reported that *T. gondii* IgG levels corresponding to the upper quartile among control subjects were significantly associated with a risk for schizophrenia and that there was no significant association between any marker of infection and other schizophrenia-like disorders or affective disorders. However, they had no indication that schizophrenia incidence is closely linked to endemic variations in *T. gondii* infections or any other risk factor. Hinze-Selch et al. (15) suggested that *T. gondii* infection, predominantly in individuals with schizophrenia, is a significant environmental factor in the interaction between psychiatric vulnerability, genetic background, immunomodulation, and the neurotransmitter structures.

In the present study, although significant differences were not found between the seropositivity of FES patients and controls, FES patients had significantly more toxoplasmosis-like symptoms; came into contact with cats more often; did consume more uncooked meat, unpasteurized goat's milk, and chicken eggs; had more contact with soil; and were living less often in urban areas and more often in rural areas. In Ireland, a higher rate of *T. gondii*-infected individuals was observed in rural areas (16,17).

Torrey et al. (8), in their meta-analysis of 11 studies, showed that the combined odds ratio was 1.60 (95% confidence interval, 0.98 to 2.69), with the predicted greater variability among the studies. They also found that individuals with schizophrenia have an increased prevalence of antibodies to *T. gondii* and suggested that this, as well as genetic and environmental factors, could be associated with a large number of cases of schizophrenia.

Schizophrenia, like multiple sclerosis and Parkinson's disease, is a chronic disease of the central nervous system; as with other similar diseases, infectious agents should be considered as possible etiologic agents, especially in persons who also have an increased genetic susceptibility (18).

Our results implied that toxoplasmosis might be, in fact, a very serious but neglected public health problem. More studies are needed to show that there is an association between *T. gondii* infection and patients suffering from schizophrenia.

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