Serological survey of *Toxoplasma gondii* in schizophrenia patients referred to Psychiatric Hospital, Sari City, Iran

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**Abstract.** Schizophrenia is a severe neuropsychiatric disorder of unknown etiology. As there is little information about the association between *Toxoplasma gondii* infection and schizophrenia in Iran, we investigated the seroprevalence of *T. gondii* in these patients and compared with that obtained in control individuals in Sari City, Iran, 2009. Eighty schizophrenia patients and 99 healthy people were examined for the presence of IgG and IgM antibodies to *T. gondii* by enzyme linked immunosorbent assay (ELISA). Overall prevalence rates of anti-*T. gondii* antibodies (IgG/IgM) in case and control groups were 72.5% and 61.6%, respectively ($P>0.05$). IgG antibodies indicating chronic form of toxoplasmosis were found in 28 (35%) and 25 (25.3%) of case and control groups, respectively ($P>0.05$). IgM antibodies (acute form) were also seen in 9 (11.2%) and 11 (11.1%) of case and control individuals, respectively ($P>0.05$). The highest 10th percentile of IgG titers in schizophrenia individuals (18.8%) was significantly higher than control group (6.1%, $P=0.02$). As prevalence rate of *T. gondii* antibodies in patients with schizophrenia was high, it seems that designing a cohort study will determine the causative relationship between *Toxoplasma* infection and schizophrenia.

**INTRODUCTION**

*Toxoplasma gondii* is a protozoan parasite found worldwide (Alvarado-Esquivel et al., 2006) that infects all kinds of mammals, including cats, livestock, and human beings. In its life cycle, cats and other felids are the definitive hosts and the other warm-blooded vertebrates are intermediate hosts. Humans may become infected with *T. gondii* by eating food or drinking water contaminated with oocysts shed by cats or by ingesting undercooked or raw meat containing tissue cysts from sheep, goats, or other animals that have acquire infection from cats (Dubey, 2004; Dawson, 2005). The importance of these modes of transmission may vary in different populations (Tenter et al., 2000). Depending on eating habits and exposure to cats, up to 80% of the population may be infected with this protozoan (Tenter et al., 2000).

Human response to *T. gondii* is related to immune status of the infected person, strain of *T. gondii* and course of infection (Suzuki, 2002). *Toxoplasma gondii* invades any type of nucleated cells (Carruthers & Blackman, 2005) and persists intracellularly in brain cells including glia and neurons (Halonen et al., 1996; Fischer et al., 1997; Luder et al., 1999). Immunocompetent hosts can contain the infection with host defense involving activated T-lymphocytes. Although all immunologic mechanisms involved are not known, it is shown that interferon-gamma (IFN-γ) and the enzyme indoleamine 2,3-dioxygenase (IDO) play an important role (Daubener & Hadding, 1997; Fujigaki et al., 2003; Oberdorfer et al., 2003). Secretion of
IFN-γ from activated T helper cells induces IDO. This enzyme degrades the tryptophan, an amino acid that is needed by tachyzoite of *T. gondii*. These parasites die due to tryptophan depletion (Daubener & Hadding, 1997; Fujigaki *et al.*, 2003; Oberdorfer *et al.*, 2003).

Most of *Toxoplasma* infections are asymptomatic to mild and in some infected persons cervical lymphadenopathy, ocular disease (Alvarado-Esquivel *et al.*, 2006; Montoya & Liesenfeld, 2004); central nervous system manifestation (Bossi *et al.*, 1998) and brain abscess may occur (Silva *et al.*, 2001). In congenital toxoplasmosis the organisms may cross the placenta and infect the fetus. The symptoms of congenital toxoplasmosis include hydrocephaly, microcephaly, intracranial calcifications, damage to the retina, and mental retardation (Torrey & Yolken, 2003). With regard to neurotropism of *T. gondii*, psychiatric manifestations such as disorientation, anxiety, depression and even psychoses with schizophreniform characters are seen in 60% of immunocompromised individuals with AIDS in whom latent infections are reactivated (Arendt *et al.*, 1999). Similar psychiatric complications and meningoencephalitis can also occur in *T. gondii*-infected immunocompetent human hosts (Carme *et al.*, 2002; Kaushik *et al.*, 2005), and human studies revealed that latent toxoplasmosis may cause personality changes (Flegr *et al.*, 1996) and decreased IQ (Flegr *et al.*, 2003). Although a potential link between *T. gondii* and neuropsychiatric disorders, particularly schizophrenia, has been proposed (Webster *et al.*, 2006), controversial results have also been reported. In north of Iran about 55.7% (Ghorbani *et al.*, 1978) of people are seropositive for *T. gondii* and there is no information about association between *T. gondii* antibodies and schizophrenia. In this study we examined the prevalence of antibodies to *T. gondii* in individuals with schizophrenia and in a matched group of control subjects.

**MATERIALS AND METHODS**

### Study population

This case-control study was performed in two populations: schizophrenia patients and control group. In 2009, 80 schizophrenia patients referred to the only Psychiatric Hospital in Mazandaran Province in Sari City, northern Iran were invited to participate in this study. The patients had been diagnosed clinically by psychiatrics. The median age of the patients was 32.95±10.05 years. All patients had no family history of schizophrenia, no evidence of immunodeficiency or other immunologic abnormalities, no history of head trauma, previous meningitis / encephalitis, or brain surgery and absence of mental retardation or other disorders of the socio-demographic general nervous system. Ninety nine healthy volunteers were selected as control group. They were screened for the absence of physical and psychiatric disorders and matched to patients according to sex, socioeconomic status, and age (33.76±10.50). After matching, we verified that the case and control groups did not differ significantly with respect to these factors (*P* >0.05). The patients and control subjects were living in the urban and rural areas of Sari City, Iran.

### Ethical aspects

This study was approved by the Research Ethical Committee of Mazandaran University of Medical Sciences, Iran. All participants were provided written informed consent after the study purpose and procedures were explained.

### Collection and examination of blood samples

Five mL of blood was obtained from each of the schizophrenia patient and healthy subject by means of venipuncture, under sterile conditions. Serum was separated from whole blood by centrifugation at 1000 r.p.m. and was stored at -80°C until use. All samples were tested blind such that the person performing the assay was not aware
of the identity of the samples. The levels of specific IgG and IgM antibodies to *T. gondii* in the serum samples were measured using enzyme linked immunosorbent assay (ELISA) technique according to the manufacturer’s instructions (Euroimmunum, D-23560 Lubeck. Seekamp 31, Germany). The IgG and IgM antibody titers were read at optical density (OD) of 490 nm using automatic ELISA reader (Spectra, Molecular Devices, USA). Sera with ≥20 and 100 IU/mL were considered positive for *T. gondii* IgG and IgM antibodies, respectively. High titer was defined as a *Toxoplasma* IgG antibody titer of >100; this category represented approximately the highest 10th percentile of IgG titers for comparing individuals in our study. The moderate titer group was defined as an IgG antibody titer of 20-100 IU/mL.

**Statistical analysis**

SPSS software V.16.0 was used for statistical analysis. The relative proportions were calculated with a confidence interval of 95%. Possible associations were identified using the Chi-Square and Fisher’s exact statistical tests at a significant level of 5%.

**RESULTS**

Fifty eight (72.5%) of 80 schizophrenia patients and 61 (61.6%) of 99 control individuals were positive for anti *T. gondii* IgG / IgM antibodies indicating prevalence of *T. gondii*. Nine (11.2%) of schizophrenia individuals and 11 (11.1%) of control group were IgM+ & IgG- indicating acute form of toxoplasmosis. Twenty eight (35%) of patients and 25 (25.3%) of control group were IgG+ & IgM- indicating chronic form of toxoplasmosis. IgM+ & IgG+ (acute form) were seen in 21 (26.2%) patients and 25 (25.3%) of control group. In contrast, 22/80 (27.5%) and 38/99 (38.4%) of individuals in case and control groups were IgM- & IgG- (seronegative). There were no statistically significant differences in any of the case and control groups. The positive OD values for anti- *T. gondii* IgG and IgM antibodies were converted into international units according to the test procedure. There was no significant difference between age of disease onset, duration of illness and *T. gondii* antibody seropositivity (*P* >0.05).

Table 1 shows the seroprevalences of IgG+ & IgM- (chronic form), IgM+ & IgG- (acute form), IgG+ & IgM+ (acute form), and IgG- & IgM- (seronegative) in case and control groups according to age groups, gender and place of living. Although patients in most age groups showed higher rates of seropositivity, the significant difference was only seen in 26-30 years old age group (*P*<0.05). The prevalence of high *Toxoplasma* IgG antibody titers (>100 IU/mL) in schizophrenia patients (18.8%) was higher than those of control subjects (6.1%) (Table 2; *P*<0.05). Analyzing the inter-relationships between IgM antibody and age groups in schizophrenia patients showed that only serofrequency of IgM antibody is significantly associated with the age groups (*P*<0.05; Table 3).

**DISCUSSION**

Schizophrenia is a severe neuropsychiatric disorder of unknown cause. Although family studies show a strong genetic component to the risk of acquiring schizophrenia, epidemiological studies indicated that some cases of schizophrenia may be associated with infectious diseases (Torrey & Yolkent, 2003). Recent studies have linked schizophrenia with prenatal exposure to viruses such as influenza A, rubella, herpes simplex type 2, and polioviruses and with postnatal exposure to viral and bacterial agents causing meningitis and encephalitis (Torrey et al., 2006). Numerous studies showed that *T. gondii* is neurotrophic, with a special affinity for glia and introduced as an agent causing schizophrenia even if the toxoplasmosis is clinically unapparent (Halonen et al., 1996; Creuzet et al., 1998). In this study, prevalence of *T. gondii* in schizophrenia patients and control individuals was 72.5% and 61.6%, respectively and the difference was not
statistically significant. Saraei-Sahnesaraei et al. (2009) in a survey in Qazvin province in Iran, showed 55.3% of the schizophrenia patients and 50.9% of the control group were seropositive for IgG specific antibodies to T. gondii, and the differences were not statistically significant. Prevalence of T. gondii in both studies carried out in Iran showed the same results in both patients and control group. Some researchers such as Cook & Derrick (1961) in Australia; Garrido & Redondo (1968) in Spain; Qiuying et al. (1999) in China; Boronow et al. (2002) in the United States, and Torrey & Yolken (2003) in Ireland reported that the differences between the two groups were not statistically significant. In contrast, other researchers (Gu et al., 2001 in China; Yolken et al., 2001 in Germany; Leweke et al., 2004 in Germany; Alvarado-Esquivel et al., 2006 in a northern Mexican city; Cetinkaya et al., 2007 in Turkey) showed that the differences between the two groups were statistically significant.

Different results in various studies may be due to many reasons which include geographical conditions, using only serological tests with no DNA detection, selection of control group, source of infection (oocyst or tissue cyst), differences in genetic susceptibility, timing of the infection, different strains of Toxoplasma and consumption of anti-schizophrenia drugs. In some studies there is an association between T. gondii infection and schizophrenia. It may be because of certain circumstances how the infection occurs or because of secondary manifestation. In Spain, schizophrenia patients showed a high rate of seropositivity to T. gondii because they worked in the hospital garden that had been faecally contaminated by the hospital's cats (Garrido & Redondo, 1968). On the other hand, institutionalized schizophrenia patients may be fed undercooked meat, thereby increasing their exposure to T. gondii. Alternatively, in some instances increased T. gondii antibodies in schizophrenia patients are secondary to immune system abnormalities, such as in individuals infected with HIV, a pathogen which is a primary agent of schizophrenia, causing reactivation of T. gondii tissue cysts in different organs and generation of antibody to T. gondii which is a secondary manifestation (Torrey et al., 2006).

In our survey there was no significant association between T. gondii antibodies and schizophrenia. There are some possible reasons including the fact that more than 90% of patients in the present study received anti-schizophrenia treatment. Leweke et al. (2004) did a study on three groups including schizophrenia patients receiving anti-schizophrenia treatment, those who had never received any drug and those of the control group. They showed that the antibody levels for the treated group were intermediate between the levels of the never-treated group and those of the control group. It suggests that anti-schizophrenia medication may have decreased the antibody levels. Antipsychotics have been shown to inhabit T. gondii in cell culture (Jones-Brando et al., 2003). On the other hand, in a study of never-treated people with schizophrenia an odd ratio of 2.70 has been reported (Leweke et al., 2004). The second factor is related to the existence of cats in this study area. A study in Ireland (Stanford et al., 1990) showed that the keeping of cats as pets was common and where the prevalence of T. gondii antibodies in the general population was high, an increase in Toxoplasma antibodies in patients with schizophrenia may be less apparent. In contrast, some studies in China showed that the keeping of cats was uncommon and prevalence of T. gondii antibodies was low (Remington et al., 2001) and the higher prevalence of antibodies in patients with schizophrenia may be more apparent. The third factor is that Mazandaran Province like France and Ethiopia shows a very high seropositivity rate for T. gondii. People in these countries consumed undercooked or (rarely raw) meat but schizophrenia has not been found to be usually prevalent (Torrey et al., 2006). It suggests that transmission by eating tissue cysts in undercooked meat
is a more benign mode of infection, and there is some evidence to support this (Ledgerwood et al., 2003). Therefore, it may have less risk for the development of schizophrenia compared to the consumption of oocysts shed in cat's faeces. In addition to the above mentioned reasons, differences in neuropathogenicity of strains of *T. gondii* (Grigg et al., 2001) and the genetic susceptibility of different human beings may explain the difference or similarity of *T. gondii* antibodies in schizophrenia patients and healthy people. In this study a significant association was observed for the high category IgG antibody titers between schizophrenia patients and control group (*P*<0.05). Since antibody titers of *Toxoplasma* IgG may remain elevated for a significant period of time, an increase in IgG antibody may reflect an active primary infection, reactivation of chronic infection or a persistent immune response to a dormant infection (Brown et al., 2005). The level of IgG antibody titer correlates with both the severity and duration since infection. Therefore, antibody titers in the high category are more likely to be associated with a current or recent reactivated infection than titers in the moderate category, which have a greater probability of reflecting dormant infection (Brown et al., 2005). This was a cross-sectional study, and most of the schizophrenia patients showed chronic form of toxoplasmosis. We could not determine when the antibodies to *T. gondii* were acquired in relation to the onset of schizophrenia symptoms. As most of the studies regarding toxoplasmosis and schizophrenia have been done using serological tests and are not based on the direct detection of *T. gondii* or DNA in infected body fluids; we suggest that a cohort study using serological and molecular methods can better define the relationship between *T. gondii* infection and onset of the symptoms of schizophrenia.

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