Seroprevalence and molecular evaluation of *Toxoplasma gondii* in Schizophrenic patients hospitalized in Sistan and Baluchestan province, Southeast of Iran

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**Abstract.** Over one-third of the world's population are seropositive for *Toxoplasma gondii*. One of the important traits of *T. gondii* is its ability to alter and manipulating the behavior and personality of its intermediate host. The current study was aimed to determine the prevalence of acute and chronic toxoplasmosis in those persons suffer from schizophrenia using serological and molecular techniques. In this cross-sectional study, blood samples were taken from 118 Schizophrenia patients hospitalized in Sistan and Baluchestan province, southeast of Iran. IgM and IgG anti-*Toxoplasma* antibodies were measured using enzyme-linked immunosorbent assay (ELISA). Furthermore, the presence of parasite was evaluated using nested-PCR *B1* gene. Among 118 schizophrenic patients, 48 (40.67%), 4 (3.37%) and 14 (11.86%) were tested seropositive only for IgG, only for IgM and for both of IgG/IgM. So that, total prevalence was 66/118 (55.91%). All samples were also examined using nested-PCR and *T. gondii* DNA was found in 41 (34.74%) samples. Our study showed high seroprevalence of toxoplasmosis in southeast of Iran.

**INTRODUCTION**

Toxoplasmosis is a cosmopolitan zoonotic disease that caused by an obligate intracellular protozoan known as *Toxoplasma gondii* (*T. gondii*) which can infect a wide range of intermediate hosts, including livestock, birds, marine mammals and humans (Zarean *et al.*, 2017; Foroutan *et al.*, 2017; Robert-Gangneux and Darde, 2012; Wang *et al.*, 2017). *T. gondii* is characterized for the first time from the liver and spleen smears of a North African rodent known as *Ctenodactylus gondii* by Nicolle and Manceaux in 1908 (Nicolle and Manceaux, 1908). Over 1/3 of the world’s population are seropositive for latent *Toxoplasma* (Foroutan-Rad *et al.*, 2016b; Robert-Gangneux and Darde, 2012). The main transmission routes for *T. gondii* are as follows: ingesting oocysts shed by infected cats, consuming raw or undercooked meat contaminated by tissue cysts, drinking water contaminated by oocysts, vertical transmission from mother to the fetus, blood transfusion, and organ transplantation.
Toxoplasma is mostly asymptomatic in immunocompetent persons, while in immunosuppressed persons can reactivate and causes some complications (Wang et al., 2017). Recently, a significant association between latent toxoplasmosis with some autoimmune diseases, neurodegenerative or mental disorders was shown (de Barros et al., 2017; Flegr, 2013; Flegr, 2015; Flegr et al., 2014; Majidiani et al., 2016; Monroe et al., 2015; Shapira et al., 2012; Sutterland et al., 2015).

Published papers have demonstrated that chronic toxoplasmosis can manipulate and alter the behavior, not only in animal models, but in men as well (Flegr, 2013; Flegr, 2015; HavlIČek et al., 2001; Khademvatan et al., 2013; Kocazeybek et al., 2009; Sutterland et al., 2015; Webster et al., 1994; Witting, 1979; Yagmur et al., 2010). Schizophrenia is a severe psychiatric and destructive mental disorder which effects on patient’s emotion, perception, personality, behavior and reduce quality of life. Also, it is considered as one of the most difficult syndromes in determination, etiology, treatment and has a wide range of clinical traits (Medeiros-Ferreira et al., 2013; Sutterland et al., 2015; Tandon et al., 2008). It is estimated that the prevalence of schizophrenia is one percent in the adult individuals and most of them suffer from the disease lifelong (Tandon et al., 2008).

In several investigations the sero-prevalence of T. gondii were determined in schizophrenia patients in the Iran country ranged between 34–72.5% (Abdollahian et al., 2017; Ahmad et al., 2010; Alipour et al., 2011; Hamidinejat et al., 2010; Khademvatan et al., 2014a; Khademvatan et al., 2014b; Saraei-Sahnesaraei et al., 2009). Due to lack of any report on schizophrenia patients in southeastern Iran, thus the current study was aimed to determine the prevalence of acute and chronic toxoplasmosis in those persons suffer from schizophrenia using serological and molecular techniques.

MATERIALS AND METHODS

In this cross-sectional study that performed between June – November 2016, blood samples were taken from 118 Schizophrenia patients who referred to three different hospitals in Zahedan and Zabol cities, southeast of Iran (Baharan Psychiatric hospital, Psychiatric clinic of Ali-ibn-Abi-Talib hospital and Amir-al Momenin Psychiatric hospital). Inclusion criteria in our study were as follows: 1) Schizophrenic patients; 2) aged ≥ 18 years old; 3) persons who tend to participate in the study (all participants signed informed consent form); 4) clinical diagnosis was done by psychiatrist following the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV-TR) criteria (First et al., 1995). From each subject who met the above-mentioned inclusion criteria of the study, five mL venous blood was collected. All blood specimens transferred to the department of Medical Parasitology of Zahedan University. At first, the taken blood was centrifuged at 4000 rpm for 5 minutes. Then sera were separated to examine the specific anti-Toxoplasma IgM and IgG antibodies and stored in -20°C. All samples were examined for IgM and IgG anti-Toxoplasma antibodies using commercial available enzyme-linked immunosorbent assay (ELISA) kit (Toxoplasma-IgG/IgM-lot No: 95001–pishtazteb Iran) based on manufacturer’s instruction by cut-off 0.262 and 0.214 for IgM and IgG, respectively. Furthermore, buffy coat separation was also done for all samples and kept in 20°C until DNA extraction for nested-PCR. DNA from each sample was extracted using DynaBio™ Blood/Tissue DNA Extraction Mini Kit (Takapoozist, Iran) based on manufacturer’s protocol and kept in -20°C till tested. Nested-PCR was employed for molecular diagnosis. For this purpose, we used two specific set primers to detect B1 gene as follows:

First stage primers
B1F1: TCAAGCAGCGTATTGTCGAG
B1R1: CCGCAGCGACTTCTATCTCT

B1F2: TCAAGCAGCGTATTGTCGAG
B1R2: CCGCAGCGACTTCTATCTCT

B1F3: TCAAGCAGCGTATTGTCGAG
B1R3: CCGCAGCGACTTCTATCTCT

B1F4: TCAAGCAGCGTATTGTCGAG
B1R4: CCGCAGCGACTTCTATCTCT
Second stage primers
B1F2 : GGAACTGCATCCGTTCATGAG
B1R2 : TCTTTAAAGCGTTCGTGGTC

Ultimately, the PCR products were electrophoresed on 1.5% agarose gel and visualized using Gel Documentation System. The findings were analyzed by SPSS software (version 19) (SPSS Inc., Chicago, IL, USA).

RESULTS

In the current study, 118 schizophrenic patients were met the above-mentioned criteria to be participated. Among them, 83.9% (N=99) were male and 16.1% (N=19) were female. The mean age of patients was 38.02 years old (age range: 19–55 y). Total prevalence was 66/118 (55.91%); so that from these, 48 (40.67%), 4 (3.37%) and 14 (11.86%) were tested seropositive only for IgG, only for IgM and for both of IgG/IgM. All samples were also examined using nested-PCR and T. gondii DNA was found in 41 (34.74%) samples Fig. 1.

DISCUSSION

One of the important traits of T. gondii is its ability to alter and manipulating the behavior and personality of its intermediate host (Flegr, 2015; Khademvatan et al., 2014a; Monroe et al., 2015; Sutterland et al., 2015). Cognitive dysfunction and behavioral changes were described in T. gondii infected-rats and mice (Webster et al., 1994; Witting, 1979). Moreover, the researchers have found that infected persons with chronic toxoplasmosis may experience and show some changes in behavior traits, personality, psychomotor skills, psychotic symptoms, etc. (Flegr, 2013; Flegr, 2015; Havlíček et al., 2001; Khademvatan et al., 2013; Sutterland et al., 2015).

In this study, the prevalence rate of T. gondii amongst schizophrenic patients was tested using both serological and molecular methods in southeast of Iran. The prevalence rate of toxoplasmosis is highly varied amongst different populations, depend upon geographical areas (Daryani et al., 2014; Foroutan-Rad et al., 2016a; Foroutan-Rad et al., 2016b; Foroutan et al., 2017). In this regards, in southeast of Iran low prevalence of T. gondii was reported in various human groups (22.7% in pregnant women, 25% in healthy blood donors, 22.8% the in general population), compared with other regions which is due to hot and dry climatic conditions that reduce T. gondii oocysts survival in the environment (Daryani et al., 2014; Foroutan-Rad et al., 2016a; Modrek et al., 2014) In this research, the overall seroprevalence to T. gondii in

Figure 1. BI-nested PCR analysis of seropositive (IgM, IgG) samples from schizophrenia patients. Lane 1) 100 bp molecular weight marker; Lane 2) negative control; Lane 3) positive control; Lanes 4 to 10) randomly selected samples from patients.
schizophrenic patients was obtained 55.9% (66/118). The current findings are very higher than recent publications in this area amongst apparently healthy individuals (Daryani et al., 2014; Foroutan-Rad et al., 2016a; Modrek et al., 2014). The IgG seroprevalence (62/118; 52.54%) of the present study is in agreement with the result of other regions in Iran (Abdollahian et al., 2017; Hamidinejat et al., 2010; Saraei-Sahnesaraei et al., 2009) and some investigation in other countries (Dogruman-Al et al., 2009). However, Some reports are in conflict with us (Ahmad et al., 2010; Alipour et al., 2011; Alvarado-Esquivel et al., 2011; Cetinkaya et al., 2007; Esshili et al., 2016; Khademvatan et al., 2014a).

Previous reports have shown a higher prevalence of anti-Toxoplasma specific antibodies in schizophrenic patients than the healthy group. In this case, Esshili et al. (74.8% vs. 53.8%; P = 0.00006; OR: 2.54 with 95% CI = 1.6–4.04) (Esshili et al., 2016), Hamidinejat et al. (57.1% vs. 29.2%; P < 0.05; OR: 2.99 with 95% CI = 1.65–5.41) (Hamidinejat et al., 2010), and Alipour et al. (67.7% vs. 37.1%; P < 0.01) (Alipour et al., 2011) reported a significant association between toxoplasmosis and schizophrenia, while some researches failed to show the same association (Ahmad et al., 2010; Khademvatan et al., 2014a). The discrepancies among studies might be originated from the study population, preciseness in sampling and selecting the participants, the variable sensitivity and specificity of some serological methods, arbitrary cutoff value or antibody titer, timing of the infection, route of infection (tissue cyst or oocyst), the heterogeneous etiology of schizophrenia disorder, genetic susceptibility, varying degrees of neuropathogenicity among various strains/genotypes of T. gondii that has distinct capability, etc. Leweke et al. suggested that anti-schizophrenia drugs reduce the anti-T. gondii specific antibody levels (Leweke et al., 2004). Besides, as previously shown, antipsychotics inhibit Toxoplasma in cell culture (Jones-Brando et al., 2003). Although, a single reason that mentioned above may have little importance on results, but they can impress the association between Toxoplasma and schizophrenia disorder. Besides, more recently a systematic review and meta-analysis paper was published by Sutterland et al. and demonstrated that chronic toxoplasmosis is not only correlated with schizophrenia (OR 1.81, P < 0.00001), but also with addiction (OR 1.91, P < 0.00001), bipolar disorder (OR 1.52, P = 0.02), and obsessive–compulsive disorder (OR 3.4, P < 0.001) (Sutterland et al., 2015). This fact may be justified with greater tendency to eat inappropriate things (pica and coprophagia), insufficient personal hygiene, low self-care skills, abnormal behaviors, so on, exposed schizophrenic patients to acquiring toxoplasmosis.

Several methods have been employed for T. gondii diagnosis, which includes skin test antigen (or so-called toxoplasmin) (STA), serological tests to assess the specific antibodies such as Sabin and Feldman test (SFT), complement fixation test (CFT), indirect haemagglutination test (IHAT), latex flocculation test (LF), indirect immunofluorescence assay (IFA), ELISA, and detection of DNA using polymerase chain reaction-based techniques (PCR) (Robert-Gangneux and Darde, 2012). Among different serological methods, ELISA has high sensitivity and specificity for diagnosis of chronic and acute toxoplasmosis (Robert-Gangneux and Darde, 2012; Saki et al., 2013). Formerly, it has been reported that IgM antibodies may be remain for a long period up to several years after acute infection. Also the presence of rheumatoid factor (RF) in serum may lead to false positive in T. gondii diagnosis; accordingly, discernment of acute and chronic T. gondii infection will be possible using IgG avidity and/or PCR technique (Robert-Gangneux and Darde, 2012; Saki et al., 2013). In the present study, in 18 out of 118 (15.25%) samples, IgM antibody was detected using ELISA, while based on nested-PCR results, T. gondii-DNA was confirmed in 41/118 (34.74%) samples that indicates acute infection of toxoplasmosis is very high in schizophrenic patients.
Current study faced several limitations, including 1) Absence of healthy individuals as control group to evaluate the possible association between latent toxoplasmosis and schizophrenia disorder; 2) No questionnaire was designed by us in this study; therefore, we were unable to assess the related risk factors including gender, residence, history of blood transfusion or transplantation, education level, ethnicity, contact with cat, source of drinking water, consumption of raw/undercooked meat, exposure to soil, etc; 3) The present investigation was based on sampling from limited participant in a limited region.

In conclusion, our study showed high seroprevalence of toxoplasmosis in comparison to previous reports in the same region. There is evidence which suggests, despite the constant presence of anti-Toxoplasma IgM antibodies in serum, any positive IgM-ELISA could not surely be considered as an acute infection, thus the PCR technique to explore the DNA of parasite is required.

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Authors’ Contributions:
M. Jafari Modrek and H. Mirahmadi conceived the study; R. Hasanzadeh performed the experiments; M. Foroutan wrote the manuscript; M. Zarean critically revised the manuscript. All authors read and approved the final manuscript.

Conflict of interests:
The authors declare no conflict of interests.

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Ethical approval:
The study protocol was approved by the Research Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran (No of Ethic code: (IR.ZAUMS.REC.1395.14). A written informed consent was obtained from all participants before blood sampling.

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