POTENTIAL ASSOCIATION BETWEEN LATENT TOXOPLASMOSIS AND SCHIZOPHRENIA SPECTRUM AND OTHER PSYCHOTIC DISORDERS

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Abstract. Studies of the potential association between latent toxoplasmosis and schizophrenia spectrum and other psychotic disorders (SSOPD) have yielded mixed results. In this study we aimed to investigate the potential association between a positive serology test for *Toxoplasma gondii* and the presence of SSOPD and selected demographic factors in order to determine if SSOPD patients should be screened for *T. gondii*. In this study, subjects were chosen from 2 groups: those with SSOPD (cases) and those without SSOPD (controls). Cases were purposely recruited from patients attending the Hospital Canselor Tuanku Muhriz, Kuala Lumpur and controls were recruited from a healthy population. The diagnosis of SSOPD was made by a mental health professional following the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). In both groups, demographic factors were recorded and each subject had blood obtained to test for indirect ELISA for *T. gondii* antibodies. A total of 218 subjects were included in the study: 109 cases and 109 controls. The prevalences of confirmed positive tests for *T. gondii* among cases and controls were 24% and 32%, respectively (p = 0.227). Those aged 18-35 years had a significantly lower seroprevalence of *T. gondii* among cases than controls (45% *vs* 70%, p = 0.001). Ethnic Malays had a significantly lower seroprevalence of *T. gondii* among cases than controls (41% *vs* 80%, p<0.001). Those with a tertiary education had a significantly lower seroprevalence of *T. gondii* among cases than controls (51% *vs* 93%, p<0.001). Unemployed subjects had a significantly greater seroprevalence of *T. gondii* among cases than controls (77% *vs* 30%, p<0.001). Those who were unmarried had a significantly greater seroprevalence of *T. gondii* among cases than controls (67% *vs* 38%, p<0.001). Those with a family history of psychiatric illness had a significantly greater seroprevalence of *T. gondii* among cases than controls (51% *vs* 6%, p<0.001). These other factors may have affected our study results. In our study, there was no significant association between toxoplasmosis and SSOPD. SSOPD patients in the study population do not need to be routinely screened for the presence of *T. gondii*. Further studies in the study population need to control for demographic factors significantly associated with positive serology for *T. gondii* in order to determine if this changes our findings.

Keywords: mental illness, chronic toxoplasmosis, *Toxoplasma gondii*, seroepidemiology, genotype

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INTRODUCTION

Toxoplasma gondii, an opportunistic parasite with world-wide distribution is capable of causing infection in humans. Domestic cats and other felines are the definitive hosts for *T. gondii*, which can excrete millions of oocysts that can survive for months in the environment (Yolken *et al*, 2017). Humans may become an intermediate host acquiring infection by ingestion of food or drink contaminated with the sporulated oocysts, ingestion of undercooked meat containing tissue cysts or via transplacental transmission (Tenter *et al*, 2000). Once a bradyzoite is ingested or a tachyzoite transmitted through the placenta to the fetus, the parasite invades tissues and organs, where it encysts and usually remains dormant for the life of the host (Carruthers and Suzuki, 2007). This lifelong infection may cause no or only mild clinical symptoms unless the immune system of the host is depressed (Pedersen *et al*, 2011; Dalimi and Abdoli, 2012).

Some studies have reported an association between latent toxoplasmosis and schizophrenia spectrum and other psychotic disorders (SSOPD) (Flegr, 2013; Karabulut *et al*, 2015; Fuglewicz *et al*, 2017). One study reported schizophrenia patients had 2.7 times greater odds of having toxoplasmosis than the general population (Arias *et al*, 2012). Another study reported up to one-fifth of schizophrenia patients had toxoplasmosis (Smith, 2014).

SSOPD includes the following disorders: schizophrenia, schizotypal personality disorder, schizophreniform disorder, brief psychotic disorder, delusional disorder, schizoaffective disorder, attenuated psychosis syndrome, substance-induced psychotic disorder, a psychotic disorder associated with a known general medical condition, catatonic disorder and other specified and unspecified psychotic disorders (American Psychiatric Association, 1994).

T. gondii has been reported to manipulate its host's behavior to benefit survival and multiplication (Vyas and Sapolsky, 2010; Sullivan and Jeffers, 2012; Długońska, 2014). Central nervous system infection with this parasite can result in increased cerebral production of dopamine (Prandovszky *et al*, 2011), a neurotransmitter in the extrapyramidal part of the brain. Several diseases are significantly associated with an abnormal level of dopamine in the brain, such as schizophrenia, Parkinson's disease, Huntington's diseases, attention deficit hyperactivity disorder (ADHD) and

addictions (Klein *et al*, 2019). Irregularities in dopamine production can lead to hyperactive transmission, eventually resulting in schizophrenic symptoms (Brisch *et al*, 2014).

Toxoplasmosis is usually diagnosed by finding parasite-specific antibodies in the plasma or serum or by finding toxoplasma DNA using a polymerase chain reaction (PCR) assay (Ivović *et al*, 2012). The nested-PCR has been reported to be the most sensitive test for diagnosing toxoplasmosis (Kong *et al*, 2012; Alghamdi *et al*, 2016; Costa *et al*, 2016).

The incidence of schizophrenia in Malaysia in 2014 was reported to be 15.2 (range: 7.7-43.0) cases per 100,000 population, being more common among males, those who live in urban areas and among migrants (Chee and Salina, 2014). Several studies from Malaysia have reported *T. gondii* IgM, IgG and IgA antibody levels are significantly higher among those with schizophrenia than those without schizophrenia (Emelia *et al*, 2012; Juanah *et al*, 2013; Chee and Salina, 2014; Omar *et al*, 2015). However, there is little epidemiological data regarding the prevalence of *T. gondii* antibodies among SSOPD patients in Malaysia. In this study we aimed to determine the prevalence of toxoplasmosis among those with and without SSOPD in the study population and determined if there was a significant association between toxoplasmosis and selected demographic factors and SSOPD in order to determine if SSOPD patients should be screened for the presence of toxoplasmosis.

MATERIALS AND METHODS

Study population

Study subjects consisted of 2 groups: those with SSOPD (cases) and those without SSOPD (controls). Cases were recruited from patients hospitalized in the psychiatry ward or attending the out-patient psychiatric clinic of the Hospital Canselor Tuanku Muhriz (HCTM) in Kuala Lumpur, Malaysia, during May-December 2018. Cases were diagnosed as having a SSOPD by a psychiatrist following the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (American Psychiatric Association, 1994). Immunocompromised patients were excluded from this study.

Controls were recruited from a healthy population by convenience sampling. A screening Mini-International Neuropsychiatric Interview (M.I.N.I.) (version 7.0.2) for SSOPD, following DSM-IV criteria, was conducted on all controls to rule out as yet undiagnosed SSOPD (Sheehan *et al*, 1997).

Each participant or legal guardian completed a standardized questionnaire that asked about selected demographic factors (age, gender, race, occupation and education level), family history of psychiatric illness and history of exposure to feline feces. Cases were asked about the duration of their mental illness, medication used to treat it and number of hospitalizations for that mental illness.

Laboratory testing

Three milliliters blood was obtained

by venipuncture from each subject, centrifuged at 3000 × g for 15 minutes and then stored at -20°C until used.

The blood obtained from each subject was examined by enzyme-linked immunosorbent assay (ELISA) for *T. gondii* antibodies and if positive was further examined by a nested-PCR assay for the presence of *T. gondii* DNA as described below. A positive nested-PCR assay sample was further examined for DNA sequence analysis.

Serological assay

Commercial indirect quantitative enzyme-linked immunosorbent assay (ELISA) kits (PLATELIA™ TOXO IgM and PLATELIA™ TOXO IgG; Bio-Rad, Marnes-la-Coquette, France) were used to measure *T. gondii* IgM and IgG antibody levels. Each test was performed in duplicate following the manufacturer's instructions. The optical densities (OD) at 420 nm and 650 nm were read using a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, Waltham, MA) to detect IgM and IgG antibody levels, respectively. A test result was considered negative for anti-*T. gondii* IgG antibodies if the level was <6 IU/ml, borderline if level was 6-8.9 IU/ml, mildly positive if the level was 9-60 IU/ml and strongly positive if the levels was >60 IU/ml. The anti-*T. gondii* IgM antibody result was interpreted as a ratio of the OD divided by the cut-off (CO) level. A ratio <0.8 was considered negative, a ratio of 0.8-1.0 was considered equivocal and a ratio >1.0 was considered positive.

Nested-PCR assay

The nested PCR test to detect *T. gondii* DNA was performed as described previously (Costa *et al*, 2016). DNA templates were extracted using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions and DNA purity was determined using a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The extracted DNA was stored at -20°C after assessing its integrity using SYBR Safe stain (Invitrogen, Carlsbad, CA) in agarose gel. The two steps of the nested-PCR assay were performed using the TopTaq Master Mix Kit (QIAGEN, Hilden, Germany) in 20 µl reactions containing 7 µl of template DNA, 0.5 µM of each primer, 10 µl TopTaq Master Mix, 2x (1.5 mM $MgCl_2$, 1.25 U TopTaq DNA polymerase, 200 µM of each dNTP) and 2 µl of 10x CoralLoad concentrate. The initial PCR reaction was performed using the primer pair GRA7FE/GRA7RE while the nested-PCR used the primer pair GRA7FI/GRA7RI. The PCR product was evaluated in 1.5% agarose gel stained with SYBR Safe. Each reaction was conducted in triplicate.

DNA sequence analysis

The PCR product of a positive sample was submitted for purification and sequencing using a HiYield™ Gel/PCR DNA Extraction Kit (Real Biotech Corp, Taipei, Taiwan) and an automatic sequencer ABI 3730XL DNA Analyzer (Applied Biosystems, Waltham, MA), respectively. The sequence was then analyzed using sequence alignment editor software, BioEdit, version 7.2.5 (Hall, 1999). The BLAST program from the US National Center for Biotechnology Information (NCBI) site was used for genotyping analysis.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 5.03 (GraphPad Software Inc, San Diego, CA). Potential associations between the groups were determined using Chi-square test, Fisher exact test or Mann-Whitney U test, where appropriate. A *p*-value <0.05 was considered statistically significant.

Ethical consideration

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee, the National University of Malaysia (UKM PPI/111/8/JEP-2018-281). All subjects, or their legal guardians for those unable to give consent, gave written informed consent prior to inclusion in the study.

RESULTS

Demographic characteristics of the study participants

The median (interquartile range (IQR)) ages of cases and controls were 38 (29-43) and 31 (25-37) years, respectively ($p = 0.026$). The majority of cases had a secondary level education (51%) while the majority of controls had a tertiary education level; this difference in education levels was significant ($p<0.001$). Significantly more cases (77%) than controls (30%) were unemployed ($p<0.001$). Significantly more cases (67%) than controls (38%) were single ($p<0.001$). Significantly more cases (51%) than controls (6%) had a family history of a psychiatric illness ($p<0.001$). There were no differences between cases and controls in the proportions who were male and in the proportions who had a cat as a pet (Table 1).

Laboratory diagnosis

Twenty-four percent of cases and 32% of controls had a positive anti-*T. gondii* IgG antibody test ($p = 0.227$) (Table 2). None of the cases or controls had a positive anti-*T. gondii* IgM antibody test. Seventeen percent of cases and 21% of controls had a low anti-*T. gondii* IgG antibody level (9-60 IU/ml) ($p = 0.489$). Seven percent of cases and 11% of controls had a high anti-*T. gondii* IgG antibody level (> 60 IU/ml) ($p = 0.482$).

The prevalence of positive anti-*T. gondii* IgG antibody test was 39% among cases with schizophrenia and comorbid substance use disorder, 29% among cases with schizophrenia and 20% among cases with substance-induced psychotic disorder.

The prevalence of a positive anti-*T. gondii* IgG antibody test in cases was 12% among subjects aged 18-35 years, 31% among subjects aged 36-55 years and 46% among subjects aged ≥56 years. Age group was significantly difference between cases and controls ($p = 0.006$). Significantly fewer ethnic Malays were cases (24%) than controls (35%) ($p = 0.003$). Significantly fewer subjects with a tertiary education were cases (16%) than controls (32%) ($p<0.001$). Significantly fewer subjects with a job were cases (28%) than controls (38%) ($p<0.001$). Significantly more subjects with a family history of psychiatric illness were cases (26%) than controls (50%) ($p<0.001$).

Table 1

Socio-demographics of SSOPD cases and healthy controls

Variables	Cases (N = 109)	Controls (N = 109)	*p*-value
Case diagnosis, *n* (%)			
Schizophrenia	73 (67)		
Schizophreniform disorder	3 (3)		
Delusional disorder	4 (4)		
Schizoaffective disorder	16 (15)		
Substance-induced psychotic disorder	5 (5)		
Schizophrenia comorbid substance use disorder	8 (7)		
Age in years, median (IQR)	38 (29-43)	31 (25-37)	0.026
Age groups in years, *n* (%)			
18-35	49 (45)	76 (70)	0.001
36-55	49 (45)	27 (25)	
≥56	11 (10)	6 (5)	
Gender, *n* (%)			
Male	48 (44)	44 (40)	0.681
Female	61 (56)	65 (60)	
Race, *n* (%)			
Malay	45 (41)	87 (80)	<0.001
Chinese	53 (49)	7 (6)	
Indian	9 (8)	7 (6)	
Others	2 (2)	8 (7)	
Education level, *n* (%)			
Primary school	10 (9)	1 (1)	<0.001
Secondary school	55 (51)	7 (6)	
Tertiary education	44 (40)	101 (93)	
Occupation, *n* (%)			
Employed	25 (23)	76 (70)	<0.001
Unemployed	84 (77)	33 (30)	
Marital status, *n* (%)			
Single	73 (67)	41 (38)	<0.001
Married/divorced	36 (33)	68 (62)	

Family history of psychiatric illness, *n* (%)			
Yes	55 (51)	6 (6)	<0.001
No	54 (49)	103 (94)	
Have a cat as a pet, *n* (%)			
Yes	34 (31)	46 (42)	0.122
No	75 (69)	63 (58)	

SSOPD: schizophrenia spectrum and other psychotic disorders; n: number of seropositive individuals; N: total number of tested individuals; IQR: interquartile range

Table 2

Evaluation of associations between selected variables and SSOPD cases or healthy controls

Variables	Cases	Controls	*p*-value
T. gondii antibody levels, *n*/*N* (%)			
IgM	0/109 (0)	0/109 (0)	
IgG	26/109 (24)	35/109 (32)	0.227
IgG low positive (9-60 IU/ml)	18/109 (17)	23/109 (21)	0.489
IgG high positive (>60 IU/ml)	8/109 (7)	12/109 (11)	0.482
Case diagnoses, *n*/*N* (%)			
Schizophrenia	21/73 (29)		
Schizophreniform disorder	0/3 (0)		
Delusional disorder	1/4 (25)		
Schizoaffective disorder	0/16 (0)		
Substance-induced psychotic disorder	1/5 (20)		
Schizophrenia comorbid substance use disorder	3/8 (38)		
Age in years, median (IQR)	41 (36-47)	33 (28-40)	0.142
Age group in years, *n*/*N* (%)			
18-35	6/49 (12)	22/76 (29)	0.006
36-55	15/49 (31)	8/27 (30)	
≥56	5/11 (46)	5/6 (83)	
Gender, *n*/*N* (%)			
Male	10/48 (21)	15/44 (34)	0.796
Female	16/61 (26)	20/65 (31)	

Race, *n*/*N* (%)			
Malay	11/45 (24)	30/87 (35)	0.003
Chinese	11/53 (21)	2/7 (29)	
Indian	2/9 (22)	1/7 (14)	
Others	2/2 (100)	2/8 (25)	
Education level, *n*/*N* (%)			
Primary school	3/10 (30)	0/1 (0)	<0.001
Secondary school	16/55 (29)	3/7 (43)	
Tertiary education	7/44 (16)	32/101 (32)	
Occupation, *n*/*N* (%)			
Employed	7/25 (28)	29/76 (38)	<0.001
Unemployed	19/84 (22)	6/33 (18)	
Marital status, *n*/*N* (%)			
Single	14/73 (19)	8/41 (20)	0.055
Married/divorced	12/36 (33)	27/68 (40)	
Family history of psychiatric illness, *n*/*N* (%)			
Yes	14/55 (26)	3/6 (50)	<0.001
No	12/54 (22)	32/103 (31)	
Have a cat as a pet, *n*/*N* (%)			
Yes	8/34 (24)	9/46 (20)	>0.999
No	18/75 (24)	26/63 (41)	

SSOPD: schizophrenia spectrum and other psychotic disorders; n: number of seropositive individuals; N: number of tested individuals in each sub-variable; IQR: interquartile range; IU/ml: international units per milliliter

A result equal or greater than 9 IU/ml was considered positive.

There were no significant differences in clinical features between cases and controls (Table 3). There was no significant association between being a case and the length of time the subject had a mental health problem (p = 0.493). There was no significant association among cases between those with and without Treatment-Resistance Schizophrenia (TRS) (p = 0.577) and between those with and without suicidal thoughts (p = 0.802).

The median (IQR) anti-*T. gondii* IgG antibody level was significantly higher among controls [1.1 (0.7-30.7)] than cases [0.7 (0.4-2.7)] (p=0.001). Similarly, significant difference in median (IQR) anti-*T. gondii* IgM antibody titer was also observed between the controls [16.1 (13.4-18.7)] and SSOPD cases [12.5 (9.2-14.5)] (p<0.001) (Fig 1).

Table 3

Clinical features of SSOPD cases with a positive anti-*T. gondii* IgG antibody test

Variables	*Toxoplasma*-seropositive patients	*p*-value
Age of disease onset, *n*/*N* (%)		
Young adults (18-35 years)	14/49 (29)	0.573
Middle-aged adults (36-55 years)	10/49 (20)	
Older adults (≥56 years)	2/11 (18)	
Duration of disease since onset, *n*/*N* (%)		
Recent onset psychosis	1/10 (10)	0.493
Ongoing history of disease	21/86 (24)	
Drug-induced psychosis	4/13 (31)	
No. of hospitalizations (severity), *n*/*N* (%)		
0-6 times	23/96 (24)	>0.999
≥7 times	3/13 (23)	
Treatment-Resistant Schizophrenia (TRS), *n*/*N* (%)		
Yes	6/21 (29)	0.577
No	20/88 (23)	
Suicidal thoughts, *n*/*N* (%)		
Yes	8/30 (27)	0.802
No	18/79 (23)	
Duration of illness, *n*/*N* (%)		
1-7 years	5/36 (14)	0.099
≥8 years	21/73 (29)	
Smoking, *n*/*N* (%)		
Yes	10/34 (29)	0.467
No	16/75 (21)	
Alcohol use, *n*/*N* (%)		
Yes	7/18 (39)	0.147
No	19/91 (21)	
Illicit drug use, *n*/*N* (%)		
Yes	5/18 (28)	0.763
No	21/91 (23)	

Medication treatment, *n*/*N* (%)		
Atypical AP	19/85 (22)	0.769
Atypical AP and mood stabilizer	3/10 (30)	
Typical and atypical AP	4/14 (29)	
Consumed AP medication during toxoplasmosis infection, *n*/*N* (%)		
Yes	9/55 (16)	0.075
No	17/54 (32)	

SSOPD: schizophrenia spectrum and other psychotic disorders; *n:* number of IgG seropositive patients; *N:* total number of patients in each sub-variable; AP: antipsychotic.

***T. gondii* detection by nested-PCR assay**

Of the 26 cases with a positive anti-*T. gondii* IgG test, only one had a positive nested-PCR assay for *T. gondii* DNA and none of the 35 controls with a positive anti-*T. gondii* IgG test had a positive nested-PCR test.

The nested PCR amplified a ~322 bp of the nest-1 product and a ~222 bp of the nest-2 product (Fig 2). Sequencing of the one positive PCR specimen revealed it belongs to the type I allele of the dense granule 7 gene (*GRA7*) of *T. gondii*, which matched with three *GRA7* gene sequences in the GenBank (accession numbers: MK250981.1 (RH strain), MH352484.1 (KS isolate), and KU599322.1 (Ankara isolate)) (Fig 3).

DISCUSSION

In this study we evaluated the potential association between a positive anti-*T. gondii* IgG test and SSOPD. We also evaluated potential associations between selected demographic factors and SSOPD.

We found no significant association between a positive anti-*T. gondii* IgG test and SSOPD, similar to previous studies from Iran (Saraei-Sahnesaraei *et al*, 2009), China (Xiao *et al*, 2010), Brazil (Campos-Carli *et al*, 2017) and Indonesia (Muflikhah *et al*, 2018). However, other studies did find a significant association between a positive anti-*T. gondii* IgG test and SSOPD (Webster *et al*, 2006; Mahmoud and Hasan, 2009; Juanah *et al*, 2013; Jones *et al*, 2014; Kahttak *et al*, 2015; Omar *et al*, 2015; Achaw *et al*, 2019; Stepanova *et al*, 2019).

The cause for this difference is unclear. One possible cause could be timing. A previous study found fewer subjects with long standing schizophrenia had toxoplasmosis than those with recent-onset psychosis (Yolken *et al*, 2017). This may be due to antibody level changes over time. In our study, the majority of SSOPD cases had long standing SSOPD that was under treatment. Previous studies have reported antipsychotic medication can inhibit replication of *T. gondii*, eventually leading to lower anti-*T. gondii* IgG antibody levels among treated patients

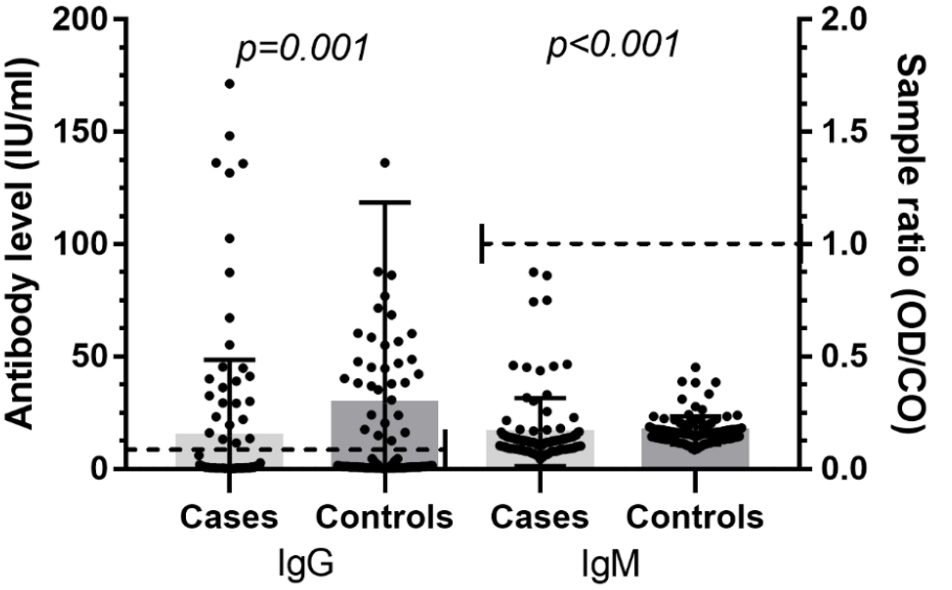

Fig 1 - Comparison of IgG and IgM antibody level between cases and controls

Horizontal dashed lines show cut-off (CO) values for IgG and IgM antibody levels and sample ratios, respectively.

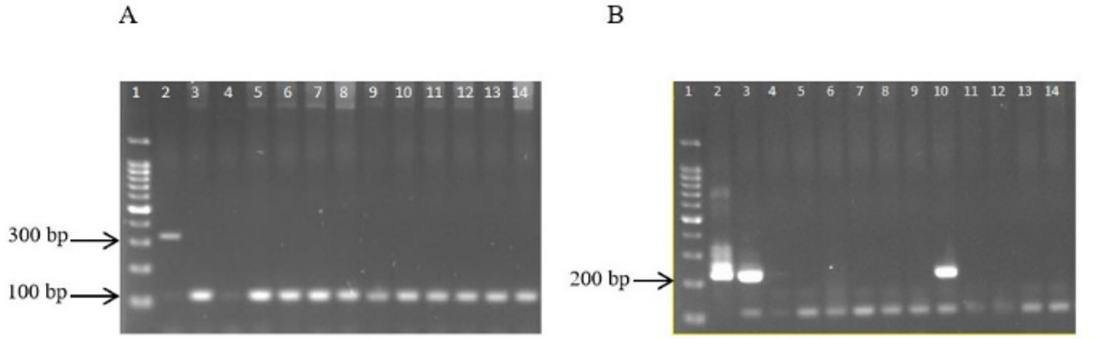

Fig 2 - PCR amplification of the *T. gondii GRA7* gene

A: Nest-1 PCR amplification; B: Nest-2 PCR amplification

Lane 1: 100 bp DNA ladder; Lane 2: Positive control 1 (DNA of *T. gondii* RH strain); Lane 3: Positive control 2 (IgG-negative blood sample spiked with DNA of *T. gondii*); Lane 4: Negative control (water); Lanes 5-11: Patient blood samples; Lanes 12-14: Control subject samples

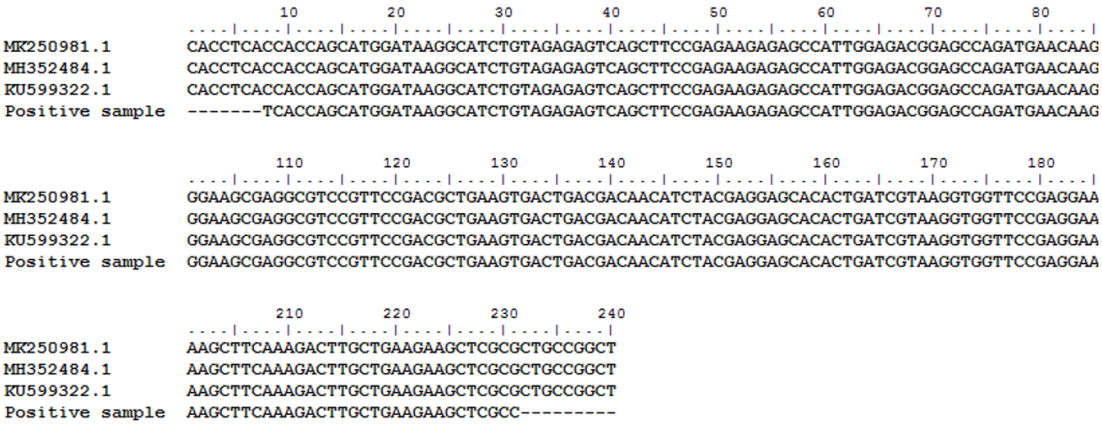

Fig 3 - Comparison of *GRA7* gene sequence isolated from a seropositive subject with the GenBank Genetic Sequence database

(Jones-Brando *et al*, 2003; Webster *et al*, 2006). It is unclear if this will cause the antibody level to drop below the lower detection level of the test resulting in a negative test. It is interesting to note that in our study, anti-*T. gondii* antibody levels among controls were higher than cases. The majority of cases were already under treatment for their SSOPD with antipsychotic drugs. A previous study reported anti-T gondii levels were higher among SSOPD patients who had not yet started treatment than those who had already been receiving antipsychotic treatment (Leweke *et al*, 2004).

Other factors may explain the difference in study results besides that discussed above. These may include the infective stage acquired, possible interactions with other infectious agents and genotypic variations. Different genotypes of *T. gondii* exhibit differences in pathogenicity and drug sensitivity (Karabulut *et al*, 2015).

T. gondii has three main genotypes, types I, II, and III, with some minor and mixed genotypes (Dardé *et al*, 1992; Cristina *et al*, 1995; Howe *et al*, 1997; Pena *et al*, 2008; Guo and Johnson, 1995; Howe and Sibley, 1995; Ajzenberg *et al*, 2004). *T. gondii* genotypes vary in prevalence, migratory capacity and ability to encyst within the host (Lindsay and Dubey, 2011; Weight and Carding, 2012; Carneiro *et al*, 2013). In our study, the *T. gondii* genotype detected was type I. *T. gondii* genotype I has been reported to be less pathogenic than genotypes II and III, which are more likely to cause chronic infections (Sibley and Boothroyd, 1992; Howe *et al*, 1996; Sibley *et al*, 2002; Webster *et al*, 2013). Tyrosine hydroxylase genes are expressed more commonly in genotypes II and III, and may be more likely to cause behavioral changes than genotype I (Gaskell *et al*, 2009;

Prandovszky *et al*, 2011). A problem in our study was that only one subject with a positive anti-*T. gondii* IgG test had a positive PCR test. This lack of data prevents us from making conclusions.

Previous studies from Malaysia reported the prevalence of anti-*T. gondii* to be in the range of 38% - 52% (Emelia *et al*, 2012; Juanah *et al*, 2013; Omar *et al*, 2015), but in our study the prevalence was 24%. The prevalence of toxoplasmosis is decreasing world-wide, possibly due to improved public awareness, food preparation and water purification (Yolken *et al*, 2017).

Karabulut *et al* (2015) reported an association between toxoplasmosis and schizophrenia depended on the *T. gondii* seroprevalence rate in the population studied. In areas with a low seroprevalence of toxoplasmosis in the population, the toxoplasmosis prevalence of those with SSOPD was significantly higher than those without SSOPD.

In our study, we found no significant association between anti-*T. gondii* among subjects with SSOPD. Further studies are needed controlling for timing, use of antipsychotics and other factors associated with SSOPD in order to determine if there is a significant association between anti-*T. gondii* and a subset population of subjects with SSOPD.

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CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

Achaw B, Tesfa H, Zeleke AJ, *et al*. Sero-prevalence of *Toxoplasma gondii* and associated risk factors among psychiatric outpatients attending University of Gondar Hospital, Northwest Ethiopia. *BMC Infect Dis* 2019; 19: 581.

Ajzenberg D, Bañuls AL, Su C, *et al*. Genetic diversity, clonality and sexuality in *Toxoplasma gondii*. *Int J Parasitol* 2004; 34: 1185-96.

Alghamdi J, Elamin MH, Alhabib S. Prevalence and genotyping of *Toxoplasma gondii* among Saudi pregnant women in Saudi Arabia. *Saudi Pharm J* 2016; 24: 645-51.

American Psychiatric Association. DSM-IV: Diagnostic and statistical manual of mental disorders. 4th ed. Arlington, VA: American Psychiatric Association; 1994.

Arias I, Sorlozano A, Villegas E, *et al*. Infectious agents associated with schizophrenia: a meta-analysis. *Schizophr Res* 2012; 136: 128-36.

Brisch R, Saniotis A, Wolf R, *et al*. The role of dopamine in schizophrenia from a neurobiological and evolutionary perspective: old fashioned, but still in vogue. *Front Psychiatry* 2014; 5: 47.

Campos-Carli SMD, Vieira ÉLM, Rocha NP, *et al*. *Toxoplasma gondii* infection and chronic schizophrenia: is there any association? *Arch Clin Psychiatry* 2017; 44-: 145-8.

Carneiro AC, Andrade GM, Costa JG, *et al*. Genetic characterization of *Toxoplasma gondii* revealed highly diverse genotypes for isolates from newborns with congenital toxoplasmosis in southeastern Brazil. *J Clin Microbiol* 2013; 51: 901-7.

Carruthers VB, Suzuki Y. Effects of *Toxoplasma gondii* infection on the brain. *Schizophr Bull* 2007; 33: 745-51.

Chee KY, Salina AA. A review of schizophrenia research in Malaysia. *Med J Malaysia* 2014; 69 (Suppl A): 46-54.

Costa ME, Oliveira CB, Andrade JM, Medeiros TA, Neto VF, Lanza DC. An alternative nested-PCR assay for the detection of *Toxoplasma gondii* strains based on *GRA7* gene sequences. *Acta Trop* 2016; 159: 120-4.

Cristina N, Dardé ML, Boudin C, Tavernier G, Pestre-Alexandre M, Ambroise-Thomas P. A DNA fingerprinting method for individual characterization of *Toxoplasma gondii* strains: combination with isoenzymatic characters for determination of linkage groups. *Parasitol Res* 1995; 81: 32-7.

Dalimi A, Abdoli A. Latent toxoplasmosis and human. *Iran J Parasitol* 2012; 7: 1-17.

Dardé ML, Bouteille B, Pestre-Alexandre M. Isoenzyme analysis of 35 *Toxoplasma gondii* isolates and the biological and epidemiological implications. *J Parasitol* 1992; 78: 786-94.

Długońska H. *Toxoplasma gondii* and the host cells. *Ann Parasitol* 2014; 60: 83-8.

Emelia O, Amal RN, Ruzanna ZZ, *et al*. Seroprevalence of anti-*Toxoplasma gondii* IgG antibody in patients with schizophrenia. *Trop Biomed* 2012; 29: 151-9.

Flegr J. Influence of latent *Toxoplasma* infection on human personality, physiology and morphology: pros and cons of the *Toxoplasma*-human model in studying the manipulation hypothesis. *J Exp Biol* 2013; 216: 127-33.

Fuglewicz AJ, Piotrowski P, Stodolak A. Relationship between toxoplasmosis and schizophrenia: A review. *Adv Clin Exp Med* 2017; 26: 1031-6.

Gaskell EA, Smith JE, Pinney JW, Westhead DR, McConkey GA. A unique dual activity amino acid hydroxylase in *Toxoplasma gondii*. *PLoS One* 2009; 4: e4801.

Guo ZG, Johnson AM. Genetic characterization of *Toxoplasma gondii* strains by random amplified polymorphic DNA polymerase chain reaction. *Parasitology* 1995; 111: 127-32.

Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999; 41: 95-8.

Howe DK, Honoré S, Derouin F, Sibley LD. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. *J Clin Microbiol* 1997; 35: 1411-4.

Howe DK, Sibley LD. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *J Infect Dis* 1995; 172: 1561-6.

Howe DK, Summers BC, Sibley LD. Acute virulence in mice is associated with markers on chromosome VIII in *Toxoplasma gondii*. *Infect Immun* 1996; 64: 5193-8.

Ivović V, Vujanić M, Živković T, Klun T, Djurkovic-Djakovic O. Molecular detection and genotyping of *Toxoplasma gondii* from clinical samples, 2012 [cited 2020 Apr 02]. Available from: URL: https://www.intechopen.com/

books/toxoplasmosis-recent-advances/molecular-detection-and-genotyping-of-toxoplasma-gondii-from-clinical-samples

Jones-Brando L, Torrey EF, Yolken R. Drugs used in the treatment of schizophrenia and bipolar disorder inhibit the replication of *Toxoplasma gondii*. *Schizophr Res* 2003; 62: 237-44.

Jones JL, Kruszon-Moran D, Rivera HN, Price C, Wilkins PP. *Toxoplasma gondii* seroprevalence in the United States 2009-2010 and comparison with the past two decades. *Am J Trop Med Hyg* 2014; 90: 1135-9.

Juanah LY, Jalaludin J, Osman M, Osman ZJ. Seroprevalence of *Toxoplasma gondii* among schizophrenics at Hospital Kajang. *Am J Infect Dis* 2013; 9: 11-6.

Kahttak A, Khan J, Rahman H. Seropositivity of *Toxoplasma gondii* latent infection in patients with neuropsychiatric disorders. *Khyber Med Univ J* 2015; 7: 14-8.

Karabulut N, Bilgiç S, Gürok MG, Karaboğa F. Is there any role of latent toxoplasmosis in schizophrenia disease? *J Chin Med Assoc* 2015; 78: 533-7.

Klein MO, Battagello DS, Cardoso AR, Hauser DN, Bittencourt JC, Correa RG. Dopamine: functions, signaling, and association with neurological diseases. *Cell Mol Neurobiol* 2019; 39: 31-59.

Kong QM, Lu SH, Tong QB, *et al*. Loop-mediated isothermal amplification (LAMP): early detection of *Toxoplasma gondii* infection in mice. *Parasit Vectors* 2012; 5: 2.

Leweke FM, Gerth CW, Koethe D, *et al*. Antibodies to infectious agents in individuals with recent onset schizophrenia. *Eur Arch Psychiatry Clin Neurosci* 2004; 254: 4-8.

Lindsay DS, Dubey JP. *Toxoplasma gondii*: the changing paradigm of congenital toxoplasmosis. *Parasitology* 2011; 138: 1829-31.

Mahmoud SS, Hasan MS. Seroprevalence of toxoplasmosis among schizophrenic patients, 2009 [cited 2020 Mar 10]. Available from: URL: https://www.ust.edu/ojs/index.php/yjmp/article/view/244

Muflikhah ND, Supargiyono, Artama WT. Seroprevalence and risk factor of toxoplasmosis in schizophrenia patients referred to Grhasia Psychiatric Hospital, Yogyakarta, Indonesia. *Afr J Infect Dis* 2018; 12 (1 Suppl): 76-82.

Omar A, Bakar OC, Adam NF, *et al*. Seropositivity and serointensity of *Toxoplasma gondii* antibodies and DNA among patients with schizophrenia. *Korean J Parasitol* 2015; 53: 29-34.

Pedersen MG, Stevens H, Pedersen CB, Nørgaard-Pedersen B, Mortensen PB. *Toxoplasma* infection and later development of schizophrenia in mothers. *Am J Psychiatry* 2011; 168: 814-21.

Pena HF, Gennari SM, Dubey JP, Su C. Population structure and mouse-virulence of *Toxoplasma gondii* in Brazil. *Int J Parasitol* 2008; 38: 561-9.

Prandovszky E, Gaskell E, Martin H, Dubey JP, Webster JP, McConkey GA. The neurotropic parasite *Toxoplasma gondii* increases dopamine metabolism. *PLoS One* 2011; 6: e23866.

Saraei-Sahnesaraei M, Shamloo F, Hashemi HJ, Khabbaz F, Alizadeh S. Relation between *Toxoplasma gondii* infections and schizophrenia. *Iranian J Psychiatry Clin Psychol* 2009; 15: 3-9. [in Persian]

Sheehan DV, Lecrubier Y, Sheehan KH, *et al*. The validity of the Mini International Neuropsychiatric Interview (MINI) according to the SCID-P and its reliability. *Eur Psychiatry* 1997; 12: 232-41.

Sibley LD, Boothroyd JC. Virulent strains of *Toxoplasma gondii* comprise a single clonal lineage. *Nature* 1992; 359: 82-5.

Sibley LD, Mordue DG, Su C, Robben PM, Howe DK. Genetic approaches to studying virulence and pathogenesis in *Toxoplasma gondii*. *Philos Trans R Soc Lond B Biol Sci* 2002; 357: 81-8.

Smith G. Estimating the population attributable fraction for schizophrenia when *Toxoplasma gondii* is assumed absent in human populations. *Prev Vet Med* 2014; 117: 425-35.

Stepanova EV, Kondrashin AV, Sergiev VP, *et al*. Toxoplasmosis and mental disorders in the Russian Federation (with special reference to schizophrenia). *PLoS One* 2019; 14: e0219454.

Sullivan WJ Jr, Jeffers V. Mechanisms of *Toxoplasma gondii* persistence and latency. *FEMS Microbiol Rev* 2012; 36: 717-33.

Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol* 2000; 30: 1217-58.

Vyas A, Sapolsky R. Manipulation of host behaviour by *Toxoplasma gondii*: what is the minimum a proposed proximate mechanism should explain? *Folia Parasitol (Praha)* 2010; 57: 88-94.

Webster JP, Kaushik M, Bristow GC, McConkey GA. *Toxoplasma gondii* infection, from predation to schizophrenia: Can animal behaviour help us understand human behaviour? *J Exp Biol* 2013; 216: 99-112.

Webster JP, Lamberton PHL, Donnelly CA, Torrey EF. Parasites as causative agents of human affective disorders? The impact of anti-psychotic, mood-stabilizer and anti-parasite medication on *Toxoplasma gondii*'s ability to alter host behaviour. *Proc Biol Sci* 2006; 273: 1023-30.

Weight CM, Carding SR. The protozoan pathogen *Toxoplasma gondii* targets the paracellular pathway to invade the intestinal epithelium. *Ann NY Acad Sci* 2012; 1258: 135-42.

Xiao Y, Yin J, Jiang N, *et al*. Seroepidemiology of human *Toxoplasma gondii* infection in China. *BMC Infect Dis* 2010; 10: 4.

Yolken R, Torrey EF, Dickerson F. Evidence of increased exposure to *Toxoplasma gondii* in individuals with recent onset psychosis but not with established schizophrenia. *PLoS Negl Trop Dis* 2017; 11: e0006040.