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SEROPREVALENCE AND RISK FACTOR OF TOXOPLASMOSIS IN SCHIZOPHRENIA PATIENTS REFERRED TO GRHASIA PSYCHIATRIC HOSPITAL, YOGYAKARTA, INDONESIA

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Abstract

Background: Toxoplasmosis is an infectious disease caused by protozoan parasite called *Toxoplasma gondii*. *Toxoplasma gondii* is an intracellular protozoan parasite belong to phylum Apicomplexa, is an obligate parasite in mammals. The active proliferating trophozoites or tachyzoites are usually seen in the acute stage of infection, while the resting bradyzoites formed tissue cysts are primary found in muscle and brain. Human infection occurs mainly by ingesting food or water contaminated with oocyst or eating an undercook meat containing tissue cyst. Human might be infected via blood transfusion, organ transplantation or transplacenta transmission. Schizophrenia is a complex neuropsychiatric disease of the central nervous system, which contributing to behavioral changes which may resulted in higher risk to *T. gondii* infection. The purpose of this study were to know difference of seroprevalence and risk factor of toxoplasmosis between schizophrenia group and control group.
Materials and Methods: Serum sample were collected 94 among schizophrenia patient at Grhasia Hospital and 64 normal population (control group). Antibody IgG of *T. gondii* was measured using ELISA method (Enzym Link Immnusorbent Assay) and questionnaires were used to collect risk factor data among the respondent.
Results: The seroprevalence antibody IgG of patient with schizophrenia (69.14%) higher than control group (65.625%), but not significantly different ($p>0.05$). There was an association between some of risk factor with seropositive of toxoplasmosis in both group. In schizophrenia group, risk factor that associated with toxoplasmosis are uncooked meat consumption, contact with uncooked meat and soil, handwashing habit, uncooked water consumption, and water source. In control group, risk factor that associated are having cattles/pet, undercook meat consumption, and water source.
Conclusion: This finding have shown seroprevalence of schizophrenia group higher than non-schizophrenia group and risk factor which associated with toxoplasmosis was different between two groups.

Key Words: Toxoplasmosis, risk factor, schizophrenia.

Introduction

Toxoplasmosis is an infectious disease caused by parasite infection of *Toxoplasma gondii*, an intracellular parasite that live inside the cells of the reticulo-endothelial and parenchymal cells of human and animals (mammals and birds) (Soedarto, 2011). Humans can be infected by consumption of uncooked meat, contaminated food or water, transmission from mother to fetus, blood transfusions, organ transplantations, and others. Some cases of toxoplasmosis usually have no symptoms, but in any cases caused severe symptoms, such as hydrocephalus, microcephalus, intracranial calcification, retinal damage, brain abscess, mental retardation, lymphadenopathy, and others.

The seroprevalence of toxoplasmosis was different in some countries and associated with various factors, such as age, eating habits and having cats (Tenter, 2000). The seroprevalence of toxoplasmosis in Indonesia was different in any regions, in Surabaya reached 63%, Jakarta was 75%, Yogyakarta was 61.5%,and Central Java was 62.54% (Chahaya, 2003; Kramer, 2000; Soedarto, 2010; Retmanasari, 2015). The high seroprevalence of toxoplasmosis associated with animals toxoplasmosis cases, such as cows, goats, pigs, chickens, ducks, and others. The seroprevalence in sheep and cattle in Yogyakarta was 78% and 21% (Artama *et al.*, 2008). Oocysts have become potential factor to spread *T. gondii* infection

in mammals including humans. Humans can be contaminated with oocysts which containing soil, for example consumed raw vegetables contaminated with cat feces. Toxoplasmosis cases in people occur at various ages, backgrounds and education levels. Seroprevalence increase with age, even in pregnant women, antibody titers would be increase 0.8% /year (Hokelek, 2015; Leblebicioglu, 2014). *Toxoplasma gondii* infection in human increased because people not informed about risk factor such as people's lifestyles and food consumption habits. People who cannot process information usually associated with education level and psychotic syndrome, such as mental retardation and schizophrenia.

Schizophrenia is a group of psychotic reaction that affects many areas of individual functions, including thinking and communicating, receiving and interpreting reality, feeling and showing emotions and behaving not acceptable manner by society (Isaac, 2005). Prevalence of schizophrenia according to the World Health Organization (WHO) showed that 1% of all population in the world, while in Indonesia the prevalence reached 1.7 $^{0}/_{00}$ and highest prevalence in Aceh and Yogyakarta (2.7 $^{0}/_{00}$) (Riset Kesehatan Dasar, 2013).

Schizophrenics have daily habit that may trigger an infection such as activities to keep hygiene such as bathing habit which would be difficult to do by their own. Futhermore, the activity outside maybe make the patient consume without knowing the foods were clean or not. This study aimed to determine the seroprevalence of toxoplasmosis and the risk factors associated with the seroprevalence in schizophrenia's population.

Materials and Methods

Study Population

Samples were collected from Grhasia Psychiatric Hospital Yogyakarta for schizophrenia group, and samples for control group obtained from the research of Prof. drh. Wayan Tunas Artama PhD. In this study, 94 schizophrenia patients were selected randomly when they were came for follow up at the psychiatry clinic between January 2015 and Maret 2015 at Grhasia Psychiatric Hospital Yogyakarta, Indonesia. They were followed screening tests such as diagnosed clinically with schizophrenia by experienced psychiatrists, came to hospital with parents or others family, communicable, and both of parents or family and patients signed the informed consent. We excluded patients who were immunocompromised including patients on chemotherapy, came to hospital without parents or family and uncommunicable patient. The risk factor data taken using questionnaires filled by patient and family to confirmed. The control group consisted of 64 healthy volunteers from Central Java Province.

Ethical aspects

This research was approved by The Ethical Committee, Faculty of Medicine, Universitas Gadjah Mada, Indonesia and Ethical Committee of Grhasia Psychiatry Hospital. The study purpose was explained to all patients and healthty volunteers before written informed consent were provided.

Collecting and analyzes samples

Materials used in the study were serum samples of schizophrenia patients and non-schizophrenia patients, ELISA kit (GenWayBioTechInc, SanDiego) which consist of microplate (coated with purified *T. gondii*) and ELISA reagents (Sample Diluent, Wash Buffer, HRP-Conjugate, TMB-substrate, HCl (Stop Solution), High Control, Low Control, Calibrator 1, Calibrator 2 Calibrator 3, and Calibrator 4), ELISA Reader, microtube, refrigerator (-20^{0}C and 4^{0}C), centrifuges, syringe, micropipettes, mikrotips, waterbath and others. The collection of blood samples carried out by competent health worker. Blood samples were taken 3 ml and centrifuged, then serum transferred into a microtube and stored in the refrigerator -20^{0} C.

IgG antibody measured using ELISA kit (Genway Biotech Inc., SanDiego) and following the steps in manufacture instruction. The preparation stage includes diluted the sample with Sample Diluent 1:40 (5 µL sample added 195 µL sample diluents). A hundred µl samples (1:40 dilution) was added to wells from A1 until H11, while calibrator 1 on B12, calibrator 2 on C12, calibrator 3 on D12, calibrator 4 on E12, High Control onF12, Low Control on G12 and blank on the A12 and H12. Then, incubated at 37^{0} C for 30 minutes. After incubation, the plate was washed 5 times using wash buffer. Then 100 µL *Horseradish Peroxidase* (HRP-conjugate) added in each wells and mix for 10 seconds and incubated at 37^{0}C for 30 minutes.After incubated, the plate washed 5 times using wash buffer. Added 100 µL of TMB-substrate to each wells, and incubated for 15 minutes in 37^{0}C.The reaction was stopped by adding 100 µL 1N HCl stop solution. Optical Density readed by ELISA Reader at 450 nm.

Data Analysis

The seroprevalence of toxoplasmosis based on the ELISA and Optical Density interpreted as positive and negative for antibodies IgG anti-*T. gondii*. Seroprevalence measured by frequency of positive subjects compared with total subjects. Risk factor was analysis using bivariate (Chi Square test) to determined which factors have relationship with seropositive of

toxoplasmosis in people with schizophrenia (p <0.05). Risk factors that have p value <0.05 was analyzed by multivariate analysis to known risk factors associated with seropositive of toxoplasmosis.

Results

Seroprevalence of Toxoplasmosis

Ninety four of schizophrenia sera and 64 non-schizophrenic were tested by Indirect ELISA and optical density from each sample compared with four calibrators and control. Analysis both groups were performed using Chi-Square statistical analysis to determined differences between two groups. (TABLE 1).

As shown in Table 1, 69.14% (65/94) of schizophrenia group and 65.625% (42/64) of non-schizophrenia group were positive for anti-*T. gondii* IgG antibody. There was no statistically difference between both groups, although the percentage of anti-*T. gondii* IgG antibody of schizophrenia group was higher than the non-schizophrenia group (psychiatrically healthy volunteers). Serointensity of anti-*T. gondii* IgG antibodies (32 IU/ml – 100 IU/ml) in schizophrenia patients (86.15%) was higher than non-schizophrenia (80.95%). Meanwhile, high positive anti-*T. gondii* antibody titer in non-schizophrenia (19.05%) was higher than schizophrenia patients (13.85%).

Table 1. Seroprevalence of anti-*T. gondii* IgG antibodies in schizophrenia patients and non-schizophrenia.

Toxoplasmosis	Schizophrenia Group (n=94)		Non-schizophrenia Group (n=64)		*Chi-square*	*p value*
	N	%	N	%		
Positive	65	69,14	42	65,625	0.261	0.642
Negative	29		22			

Table 2. Serointensity of IgG Antibodies Anti- *T. gondii* in Schizophrenia Group and Non-Schizophrenia Group

Concentration of Antibody IgG	Schizophrenia (n-65) (%)	Non-schizophrenia (n-42) (%)
Low positive antibody titer (32 IU/ml – 100 IU/ml)	56 (86.15)	34 (80.95)
High positive antibody titer (> 100 IU/ml)	9 (13.85)	8 (19.05)

Association of Risk Factor with Seroprevalence of Toxoplasmosis

The association of risk factors in schizophrenia patients and toxoplasmosis cases indicated by characteristics which statistically affected (p value≤0.05), include grilled meat (0.019), contact with raw meat/soil (0.050), handwashing habit (0.002), water sources (0.033) and uncooked water consumption (0.019). Characteristics were considered in non-schizophrenia group are having pet (0.001), uncooked meat (grilled meat) (0.036), and water sources (0.023). Characteristics of schizophrenia consist of disease duration, activity outside the house, and bathing habits only tested in schizophrenia group. Adding characteristics in schizophrenia group aims to more explore the possibility risk factors which potentially associated with daily activities of schizophrenia patient. (TABLE 3)

Risk factor has higher odds ratio (OR) in schizophrenia group is consumption of uncooked water (10.471), then following by water source (3.209), contact with uncooked meat/soil such as cooking, farming and gardening with OR 0.570 and consumption of meat grilled with OR 0.299, and handwashing habits with OR 0142. Risk factor have significant relationship with seroprevalence in the non-schizophrenia group with the highest OR is having pet/livestock (9.403), and odds ratio from another characteristics are consumption of meat grilled (0.323) and water sources (0.241).

Table 3: Statistical Analysis of Association of Environmental Characteristics and Seropositive of Toxoplasmosis

No	Characteristics	Schizophrenia		Non schizophrenia	
		Odd Ratio (CI 95%)	*p value*	*Odd Ratio* (CI 95%)	*p value*
1	Consumption of beef satay	1,091 (0.444-2.682)	0,850	1,000 (0.356-2.806)	1.000
2	Consumption of goat satay	0.958 0.388-2.354)	0,926	1.493 0.476-4.689)	0,491
3	Consumption of chicken satay	2.333 (0.312-17.434)	0,397	0.950 (0.160-5.643)	0,955
4	Consumption of grilled meat	0.304 (0.109-0.846)	0,019*	0.310 (0.101-0.947)	0,036*
5	Consumption of rabbit satay	1.136 (0.325-3.975)	0,841	0.567 (0.151-2.120)	0,395
6	Consumption of raw vegetables	0.978 (0.397-2.408)	0,961	2.053 (0.378-11.142)	0.397
7	Having pet	0.787 (0.301-2.059)	0,625	10.291 (2.621-40.401)	0,001*
8	Contact with uncooked meat/soil	0.395 (0.148-1.054)	0,050*	1.731 (0.587-5.106)	0,318
9	Activities outside	1,029 (0.409-2.587)	0,952	-	-
10	Bathing habit	0.978 (0.397-2.408)	0,961	-	-
11	Handwashing habit	0.157 0.149-1.167)	0,002*	0.381 (0.104-1.419)	0,135
12	Water sources	3.423 (1.061-11.045)	0,033*	0,281 (0.092-0.862)	0,023*
13	Consumption uncooked water	8.400 1.053-66.988)	0,019*	1,054 (0.421-3.161)	0,982

*Show p value < 0.05, it have association with toxoplasmosis

Table 4: Binary Logistic Regression Analysis of Risk Factors in Schizophrenia Group

No.	Characteristics	OR
1	Consumption of grilled meat	0.299
2	Contact with uncooked meat.soil	0.570
3	Water sources	3.209
4	Consumption uncooked water	10.471
5	Handwashing habit	0.142

Table 5: Binary Logistic Regression Analysis of Risk Factors in Non-Schizophrenia Group

No.	Characteristics	OR
1	Having pet/livestock	9.403
2	Consumption of grilled meat	0.323
3	Water sources	0.241

Discussion

Toxoplasmosis is a common disease caused by *Toxoplasma gondii* infection in various ways, such as according to environment, eating habits, contact with this obligate intracellular protozoan occurs through direct ingestion of food or water contaminated with cat feces containing oocysts, ingestion of tissue cysts in uncooked meat, transplacental infection of the fetus, blood transfusion or organ transplantation. Our data show the prevalence of *T. gondii* antibodies in individuals with schizophrenia is no significantly different with the control populations (*p value* 0.642). High seroprevalence of toxoplasmosis determined if more than 40%, so seroprevalence in both group were high, in schizophrenia group reached 69.14% and control group reached 65.625%. This data supported by other studies which found higher levels of antibody titers in schizophrenic patients than in the control group, such as Ahmad Daryani *et al*. (2010) in Iran showed 35% of

schizophrenia patients and 25.3% of the control group were seropositive for anti-*T. gondii* IgG antibody and the difference was not statistically significant and Saraei-Sahnesaraei *et al*. (2009) showed 55.3% of the schizophrenia patients and 50.9% of the control group were seropositive for IgG specific antibodies to *T. gondii*. Emelia *et al* (2012) also showed higher seropositive in schizophrenia group (37.5%) than control group (34%) and was not statistically different. These findings result have many reasons such as the difference in control group selection,difference in genetic susceptibility and antipsychotic drugs consumptions. Antipsychotic drug have been observed in schizophrenia treatment to inhibit tachyzoite replication in cell culture (Goodwin *et al*, 2011). Other researcher found the difference effect of antipsychotic drug in schizophrenia patients. Schizophrenia patients who treated with antipsychotic drug showed level of Anti-*T.gondii* IgG antibody higher than control group (non schizophrenia) and lower than schizophrenia patients without antipsychotic treatment (Leweke *et al*., 2004). Treatment effect of antipsychotic drug on Tg antibody not clear yet, but some study related to an elevated of time period with Tg exposure or treatment effect of microbial replication (Emelia *et al*., 2012). In fact, more than 90% of schizophrenia patient (subject) received anti-psychotic drug to decrease the schizophrenia symptoms and could be the one possible reason for not significant result.

Recent studies show linked schizophrenia with prenatal exposure to pathogen such as influenza A viruses, rubella viruses, herpes simplex viruses type 2, and polio viruses and with postnatal exposure to viral and bacterial agents causing meningitis and encephalitis (Torrey *et al*., 2012). High seropositive anti-*T.gondii* antibodies in schizophrenic patients was associated with higher inflammatory responses (Hinze-Selch *et al*., 2007), whereby interferon gamma (IFN-γ) and indoleamine 2,3-deoxygenase (IDO) enzyme play an important role. Induction of IFN-γ controlling *T. gondii* infection in the CNS, preventing tachyzoite replication, cyst formation and toxoplasmic encephalitis.

The *T. gondii* infection stimulate the production of various cytokines by microglia, astrocytes, and neurons cell which initiated inflammatory responses (Lambert *et al*., 2006; Chao *et al*., 1993; Fishcer *et al*., 1997; Halonen *et al*., 1998; Jones *et al*., 2003; Schwartzman *et al*., 1987). The cysts formed within the brain and produce tyrosine hydroxylase, which needed for dopamine production, by convert L-Dopa to dopamine. Indoleamine 2,3 deoxygenase degrades the tryptophan, which needed for the replication of tachyzoite (Lambert *et al*., 2006; Chao *et al*., 1993; Fishcer *et al*., 1997). Tryptophan degradation products accumulate via the kynurenine pathway (Miller *et al*., 2004) and have effect for excess dopaminergic activity. The impact of dopaminergic activity produce less serotonin as seen in schizophrenia with accumulation of dopamine. *T. gondii* infection in brain and CNS increased levels of dopamine and caused psychotic symptoms such as schizophrenia (Ferguson *et al*., 1987; Bertoli *et al*., 1995).

Statistical analysis show some risk factor associate with toxoplamosis, in control group are having pet, consumption of grilled meat, and water sources and in schizophrenia group are consumption of grilled meat, contact with uncooked meat/soil, consumption of uncooked water, handwashing habit, and water sources. Having pet and livestock become potential risk factor because it has role of transmission. Animals can be infected from contamination of cat faeces, contamination of herbivore, and carnivore (Gangneux and Marie, 2012). In schizophrenia group, there is no correlation of having pet with toxoplasmosis, but in control group does (*p value* 0.001, OR 9.403). Consumption of uncooked infected-animal directly infect human and other animals. Peoples have various animal, such as cat, goat, sheep, chicken, duck, and fowl.

Water sources is a risk factor associated in both group. Oocyst may contaminated water sources which usually used for cooking, bathing, washing, and other activities. Contamination can spread out through water flow (rain or river) and contaminated surrounding area. Spreading contamination area increase the possibility of toxoplasmosis. Consumption of uncooked water become potential risk factor for human around contaminated water sources. Uncooked water consumption also showed significant association and could be occur directly from drinking water or accidentally when holliday activities in water area. Retmanasari (2015) found the association of consumption of uncooked water in central java province, Indonesia and have correlation with geographical condition. Consumption of uncooked water have correlation with water sources and in this study showed p value of both characteristic < 0.05, associated with toxoplasmosis. Using unfitrate water have risk for Tg infection in subject than filtrate water.

One of toxoplasmosis risk factor is eating habit, such as consumption of uncooked meat. This research proved association of grilled meat consumption with toxoplasmosis in both group (OR 0.050 and 0.570). Human activities usually associated with their job, such as a former which usually contact with soil; a chef –contact with uncooked meat and raw vegetables; meat seller; a butcher; or a contraction laborers. This study showed direct contact with raw meat and soil statistically associated with toxoplasmosis in control group. Tg infection could be occurs via skin lession or accidentally ingested.

Tg oocyst is an infective stage and can survive in many type of soil and temperature. It survive more than a year at 4^0C, 100 days at 10^0C, 32 days at 35^0C, dan 9 days at 40^0C (Gangneux and Marie, 2012). One of rehabilitation programme for schizophrenia patients in Grhasia Pscyatric Hospital is farming various vegetables. Contamination can be spread from contaminated soil with oocys, even this condition not finding yet. This patients become infected because there is less protection and showed association between toxoplasmosis and hand washing habits. Individuals with schizophrenia have less knowledge of the important to keep the individual hygenes. The significants finding of hand washing habit showed environmental contamination and unprotected patient during rehabilitation in hospital.

Association of meat consumption with toxoplasmosis in schizophrenia also found by Juannah *et al.,* (2013) in Malaysia, Alvarado-Esquivel *et al.,* (2011) in Mexico, Wang *et al.,* (2014) in China, dan Torrey *et al.,* (2012) in USA. Meat

consumption assume the contamination of uncooked meat with Tg bradizoites and unperfectly cooked, so the bradizoites could be activated become tachyzoites and detected in serology test of Tg antibody. Contamination of uncooked meat was an impact from environmental contamination with oocyst. Oocyst can directly throught when the livestock lived and becoming passive form to avoid the immune defense. Toxoplasmosis's prevalence in animal play role of transmission in human. Artama *et al* (2008) showed high Tg-infected animal in Yogyakarata reached 78% in sheep and 21% in cattle.

Ages, gender, and consumption of raw vegetables have no correlation with toxoplasmosis in both group. Althought, some research explained highest seropositive rate for Tg antibody in age over 40 years old (Emelia *et al.*, 2012). Gender have no correlation too and we assume it could be dependent of individual activities and habits factors. Consumption of raw vegetables and fruits not associated because most subjects consume it after washing and peeling the fruit. Bathing habits and activities outside are spesific characteristics which allowed in schizophrenia group and showed no correlation. This findings described their families care enough for their hygenes attitude and not allow patients went outside alone.

Conclusion

Seroprevalence of IgG antibodies anti-*T. gondii* in schizophrenia group was higher than the non-schizophrenia group. Risk factors of toxoplasmosis which have association in schizophrenia group are consumption of meat grilled/roasted, contact with uncooked meat/soil, consumption of uncooked water, hand washing habits and water sources. While the risk factors that have been associated non-schizophrenia group are, having pets/livestock, consumption of meat grilled/roasted and water sources.

Conflict of Interest: The authors state that they have no conflict of interest**.**

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