

# Seroprevalence of *Toxoplasma gondii* Infection in Patients with Alzheimer's Disease

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Received 2016 February 18; Revised 2016 June 10; Accepted 2016 June 12.

## Abstract

**Background:** Toxoplasmosis is one of the most important diseases in humans and animals. Almost one-third of the human population around the world is infected with toxoplasmosis. The agent of this parasitic disease is a protozoan called *Toxoplasma gondii* (*T. gondii*) that causes encephalitis in people with suppressed immune systems and abortion, mental retardation and chorioretinitis in the fetus. Alzheimer's disease (AD) is the most important neurodegenerative disease.

**Objectives:** Due to the high prevalence of toxoplasmosis in Iran and evidence on its impact on neurodegenerative diseases, this study was performed to evaluate the *T. gondii* infection in patients with AD.

**Patients and Methods:** In this case-control study, after selection of alzheimer's patients (APs) referred to Imam Reza psychiatric hospital of Khorramabad, west of Iran, and healthy controls (each group consisted of 87 individuals), using the convenience sampling method and under the supervision of a neurologist, blood samples were taken during July 2014 and January 2015. The collected samples were transferred to the research laboratory of parasitology under cold chain storage and then, the serum samples were separated by centrifugation and were frozen at -20°C until use. The *T. gondii* IgM and IgG specific antibodies were assessed in serum samples using commercial Enzyme Linked Immunosorbent Assay (ELISA) kits.

**Results:** The overall prevalence of *T. gondii* infection in patients with AD and the control group, using ELISA assay, was obtained as 66.6% (58/87) and 56.32% (49/87), respectively ( $P = 0.99$ ). In this study, there was no significant association between *T. gondii* infection and AD. On the other hand, no statistically significant difference was observed between the two groups in terms of infection with *T. gondii* ( $P = 0.99$ ).

**Conclusions:** Higher prevalence of *T. gondii* in patients with AD compared to controls showed the possible impact of this parasite in AD, which may exacerbate symptoms, and this requires special attention of specialists and patient families.

**Keywords:** Seroprevalence, *Toxoplasma gondii*, Patients with Alzheimer's Disease, Control Group

## 1. Background

Toxoplasmosis is a zoonotic disease caused by an obligate intracellular parasite, and routinely occurs through eating raw or undercooked infected meat, transfer of placental blood transfusion or organ transplant (1, 2). In most people with a healthy immune system, primary or chronic *Toxoplasma gondii* (*T. gondii*) infection has no clinical symptoms. Toxoplasmosis is particularly important in people with an impaired immune system, transplant patients and pregnant women (3, 4). Acquisition of infection occurs by eating raw/undercooked meat containing tissue cysts or food and water infected with oocysts excreted by a cat. Therefore, in areas where people eat more raw/undercooked meat or where cats are more closely in contact with humans, the rate of infection is higher (5).

Regularly, this parasitic infection is asymptomatic or has mild symptoms such as; eye problems and lymphadenopathy creates. The most common test for detection of *T. gondii* infection is the Enzyme-Linked Immunosorbent Assay (ELISA) technique, which is available as commercial kits. This technique detects parasite-specific IgM and IgG antibodies in human serum (6). Approximately one-third of the world's human population is infected with *T. gondii*. The released parasites go through different parts of the host body and enter all nucleated cells of the body, especially cells of the nervous system, which causes necrosis and creates massive lesions (7-10). Most immunocompetent individuals carrying the parasite cysts, especially in the central nervous system, have a model of chronic asymptomatic common infection (11, 12). There are several

reports that show chronic *Toxoplasma* infection may alter human behavior. Behavioral changes attributed to infection due to *T. gondii* may be due to the parasite itself, which causes infection with nerve damage. IFN- $\gamma$ , the main responsible cytokine for immunological defense against *T. gondii* is essential in all infected tissues, including the central nervous system (13-17). The biosynthesis of dopamine and/or serotonin could be directly affected by aromatic amino acid hydroxylases in *T. gondii* genome. Increase of dopamine in the brains of infected patients can be associated with the missing link between toxoplasmosis and mental disorders (15, 18). Although, latent cases treated as asymptomatic and clinically unimportant by medical professionals, results of animal studies and recent studies of personality profiles, behavior, and psychomotor performance have led to reconsider *T. gondii* infection as a risk factor for the development of some neuropsychiatric disorders, including schizophrenia, parkinson disease (PD) and alzheimer's disease (AD) (10, 13, 18, 19).

Alzheimer's disease is a progressive slow neurodegenerative disorder that is the most important and common cause of dementia in the elderly and is considered to normally occur at an older age. In this neurodegenerative disease, recent memory is severely damaged but the memory of past events, remains relatively healthy (20-22). The neurofibrillary filaments in brain cells, as well as the formation of amyloid plaques around nerve cells, are the main causes of AD (23). The clinical characteristics of AD include cognitive impairment forces, general decline in performance, disorder in the ability to perform daily living activities and behavioral disturbance (24). Injuries to the brain caused by plaque formation, including the release of the acetyl cholinesterase enzyme of plaques and toxic effects of amyloid deposits in brain cells, lead to the creation of inflammation in brain tissue. Neuro-filaments due to hyper-phosphorylation of Tau protein and the discontinuity of the skeletal structure of neurons that role phosphorylated state of Tau protein, maintaining the stability of microtubules constituent neural pathways (25, 26). Alzheimer's disease consists of three clinical stages including; mild, moderate and advanced. Early symptoms of AD gradual disruption memory, especially space memory that reduces the amount of acetylcholine as a result of the high release of the acetyl cholinesterase enzyme, which is the cause of Alzheimer's, impairs spatial memory (27). Some researchers have shown that the transport of oxygen to the brain and decrease in blood stream may act as a mechanism in the complex etiology of AD (28). This study was conducted with regards to the localization of *T. gondii* tissue cysts in the central nervous system and reports indicating the impact of *T. gondii* on neurodegenerative diseases (7, 9, 10, 13, 16, 29, 30), as well as the lack of information

about the prevalence of toxoplasmosis in patients with AD in studies conducted on the prevalence of parasitic infections in Lorestan province (18, 19, 31-37).

## 2. Objectives

This study aimed to evaluate *T. gondii* infection in patients with AD.

## 3. Patients and Methods

### 3.1. Study Population

In this case-control study, the study population consisted of 87 alzheimer's patients (APs) and 87 healthy control subjects, who were selected using the convenience sampling method. To determine the sample size based on the statistical formula and with regards to  $P_0 = 0.35$ ,  $P_1 = 0.55$ ,  $\alpha = 0.05$  and  $\beta = 0.20$ , the sample size was calculated as 105 patients in each group. Because this number of intended patients was not found, the sample size was calculated to the maximum extent possible (87 people in each group).

The APs were under the medical supervision of a neurologist at the Imam Reza psychiatric hospital of Khorramabad, west of Iran. The mental status of APs was determined by a neurology specialist, using associated factors such as navigation, registration, attention, memory, language and construction (Table 1). According to these factors, the stage of AD was defined as mild, moderate and advanced. Only individuals, who were in one of the stages of AD were included in the case group. Based on the neurologist examination and documents, the control group had no neurological and psychological problem. People who had any items related to AD or were treated with any medication for neurodegenerative disorders were excluded from the control group. After describing the study and gaining the consent of participants, demographic variables such as age, gender, stage of AD, type of food and drinking water, the level of education and place of residence were collected via a designed questionnaire. The study participants were recruited with a prior consent and they were assured that their names would not be mentioned and they would be notified about the results of the research. The ethics committees of the involved institutions approved this study (2014.3.1/200.22.45).

### 3.2. Samples Collection

Whole blood samples were taken from the APs and control group during July 2014 and January 2015. The collected samples were transferred to the research laboratory of parasitology along with ice and cold chain storage conditions,

**Table 1.** Brief Examination of Mental Status of Alzheimer's Patients

Factor Title	Score
<b>Navigation</b>	
Time (1 score for each year, quarter, month, date and day of the week)	5
Position (1 point for each State, country, city, buildings, units or rooms)	5
<b>Registration</b>	
Repeat the names of three devices (1 point for each device)	3
<b>Attention</b>	
7 to 7 to subtract from 100 to 5 times or spell out the word upside down	5
<b>Remind</b>	
Repeat three devices that have already been named (1 point for each device)	3
<b>Language</b>	
Naming pens or wristwatches (1 point each)	2
Repeating a meaningless sentence	1
Doing a three-step command (1 point for each)	3
Reading and performing the sentence "close your eyes"	1
Write a complete sentence	1
<b>Construction</b>	
Copy of the picture two intersecting pentagons	1
<b>Total</b>	<b>30</b>

and then centrifuged for three to five minutes at  $3500 \times g$ . The isolated serum was separately poured in sterile 1.5-mL micro tubes and stored at  $-20^{\circ}\text{C}$  until use.

### 3.3. Seroprevalence Evaluation

To assess the anti-*T. gondii* IgG and IgM specific antibodies in serum samples, the *Toxoplasma* IgM and IgG ELISA kits (Pishtazteb Co, Iran) were used. Firstly, each serum sample was diluted by the diluent samples provided in the kit. Other experimental stages were performed according to the manufacturer's instructions. Optical density (OD) of each sample was measured using an ELISA reader (AWARENESS, Germany) and 450 and 630-nm filters. To determine the positive and negative samples for *Toxoplasma* specific antibodies, according to the kit instructions, a number of sample indexes were obtained by dividing the sample absorbance by the amount of cut-off. Based on this, amounts higher than 1.1 were considered as positive and lower than 0.9 were considered as negative. Samples with indexes between 0.9 and 1.1 were considered as suspicious and their fresh serum samples were tested again. To avoid any bias of equipment (ELISA reader, ELISA washer and samplers)

and observer, all devices and instruments were calibrated before starting the procedures, and all experiments were done by one observer.

### 3.4. Stage and Severity of AD

In this study, the stage and severity of AD was determined according to Table 1, which is the mini mental state examination of AD to check the possibility of dementia, and total accumulate points of less than 24 were considered as a landmark for AD (although this criterion changes partly with age and education level) (24).

### 3.5. Statistical Analysis

Results of the examined serum samples along with demographic information and other collected information via the questionnaires were analyzed using the SPSS 18 software (SPSS, Chicago, IL, USA). In this study, in addition to the multivariate logistic regression, Chi-square test and Fisher's exact test were used to analyze the data. Associations were tested using odds ratios (OR) and 95% confidence intervals (CI) after adjustments. A P value of  $< 0.05$  was considered statistically significant for differences.

## 4. Results

### 4.1. Demographic Characteristics and Seroprevalence Status of the Participants

The average age of the subjects was  $21.71 \pm 61.9$  years and in terms of gender, there were 66 males (37.9%) and 108 females (62.1%). The examined variables of participants in terms of infection with AD are shown in Table 2. The seroprevalence of *T. gondii* in patients with AD and the control group was 66.6% (58/87) and 56.32% (49/87), respectively. Various titers of the *Toxoplasma*-IgG antibody were found in 107 cases (61.5%) of the total cases, which indicate the chronic phase of *Toxoplasma* infection. In none of the subjects the *Toxoplasma*-IgM antibody was found to have a relationship with the acute phase of the disease. In the present study, the chi-square test showed that there was a significant relationship between the seroprevalence of *Toxoplasma* and variables of age ( $P = 0.016$ ), gender ( $P = 0.036$ ), place of residence ( $P < 0.001$ ) and the type of diet ( $P = 0.010$ ) in both case and control groups. Therefore, the prevalence of *T. gondii* infection in residents of the city (63.8%), who had a mixed diet (92%), were older than 60 years (56.9%), females (62.1%) and tap water consumers (92%) was significantly higher than the other groups (Table 3).

**Table 2.** The Frequency of Different Examined Variables in Terms of Developing and Non-Developing Alzheimer's Disease

Variable	Disease status	
	Alzheimer Patients No. (%)	Healthy Control No. (%)
<b>Gender</b>		
Male	40 (46.0)	26 (29.9)
Female	47 (54.0)	61 (70.1)
<b>Place of residence</b>		
City	61 (70.1)	50 (57.5)
Village	26 (29.9)	37 (42.5)
<b>Type of consuming water</b>		
Piped	75 (86.2)	85 (97.7)
Well	12 (13.8)	2 (2.3)
<b>Type of diet</b>		
Mostly vegetables	0 (0.0)	0 (0.0)
Mostly protein	2 (2.3)	12 (13.8)
A mixture of both	85 (97.7)	75 (86.2)
<b>Education</b>		
Illiterate	85 (97.7)	9 (10.35)
Elementary	2 (2.3)	37 (42.53)
Cycle	0 (0.0)	12 (13.8)
Diploma	0 (0.0)	17 (19.55)
Advanced diploma	0 (0.0)	2 (2.3)
Bachelor	0 (0.0)	10 (11.5)
<b>Age (Mean <math>\pm</math> SD)</b>	78.17 $\pm$ 10.38	45.63 $\pm$ 17.45

#### 4.2. Stage and Severity of AD

In terms of stage and severity of AD, 15 (17.2%), 11 (12.6%) and 61 (70.1%) patients were at the mild, middle and advanced stage of the disease, respectively. According to the chi-square test, there was no significant relationship between the seroprevalence of *Toxoplasma* and stage of AD ( $P = 0.310$ ) (Table 3).

#### 5. Discussion

Toxoplasmosis is a zoonotic parasitic disease that can be transmitted to humans through various routes such as, food, water or soil contaminated with oocysts, raw or undercooked meat containing tissue cyst, trans-placental transmission, blood transfusion and organ transplantation (3, 38). Various techniques including indirect immunofluorescence, chemiluminescence histology, isolation of parasite, ELISA and polymerase chain reaction (PCR) have been used for detection the disease (1, 39, 40) studies have shown that the brain is one of the main localization

sites of *T. gondii* that the parasite localized in this part as the tissue cyst form. Due to the immune system response, the membrane-enclosed parasites in the brain constitute the tissue cysts. Since the brain is the primary target organ after infection with *T. gondii*, various psychiatric and behavioral disorders, as well as mental illnesses, can be associated with toxoplasmosis (2, 31, 41). So far, the central nervous system infections have been proposed as possible etiologic factors in the development of sporadic AD.18 In this study, the prevalence of *Toxoplasma*-specific IgG antibodies in the serum of APs was slightly higher than the controls (66.6% and 56.32%, respectively). This finding is partially consistent with results reported by Kusbeci et al., 2011, in which 44.1% of APs and 24.3% of controls were positive for IgG antibodies against *T. gondii* (28). Results and analysis of the data based on Chi-square and multivariate logistic regression tests in serological assays are as follows: a) with assumption of constant non-experimental variables, the risk of AD for men is almost 3.2 times more than women; according to the P value of 0.036, the meaningful relation-

**Table 3.** Crosstab of Participants Based on Different Examined Variables and Infection With *T. gondii*

Variable	Toxoplasma Serology Result		P value
	Positive No. (%)	Negative No. (%)	
<b>Gender</b>			0.036
Male	38 (21.8)	28 (16.1)	
Female	69 (39.7)	39 (22.4)	
<b>Place of residence</b>			< 0.001
City	54 (30.33)	57 (32.8)	
Village	53 (29.77)	10 (5.61)	
<b>Type of consuming water</b>			0.422
Piped	93 (53.4)	67 (38.5)	
Well	14 (8.0)	0 (0.0)	
<b>Type of diet</b>			0.010
Mostly vegetables	0 (0.0)	0 (0.0)	
Mostly protein	13 (7.30)	1 (0.56)	
A mixture of both	94 (52.80)	66 (37.07)	
<b>Education</b>			0.005
Illiterate	30 (17.2)	64 (36.8)	
Elementary	27 (15.5)	12 (6.9)	
Cycle	6 (3.4)	6 (3.4)	
Diploma	8 (4.6)	9 (5.2)	
Advanced diploma and bachelor	2 (1.1)	10 (5.7)	
<b>Age, (Mean <math>\pm</math> SD)</b>	65 $\pm$ 20.3	56.92 $\pm$ 23.1	0.016
<b>Stage of Alzheimer's disease (Case group)</b>			0.310
Primary	8 (9.2)	7 (8.0)	
Average	9 (10.3)	2 (2.3)	
Advanced	41 (47.1)	20 (23.0)	

ship that can be caused by more exposure of men with the agent of the disease; b) with the assumption of constant non-experimental variables, for each unit increase in age, the chance of developing AD increases by 19%; according to the  $P < 0.001$  this increase was significant. The increased prevalence of toxoplasmosis with aging may indicate the association of AD with toxoplasmosis (Table 4) (30). The specific treatment in children has a better prognosis than adults, while the mortality rate is lower and the duration of treatment is shorter; c) In patients with AD, the symptoms of incontinence ( $P = 0.012$ ) and change the mode ( $P = 0.014$ ) showed a significant relationship with the results of serology test that these symptoms as the risk factors involved in AD can also be related to the increased prevalence of *T. gondii*; d) The type of nutrition ( $P = 0.010$ ) and place of residence ( $P < 0.001$ ) also had a significant relationship with

the results of the serology test (Table 3).

According to the results of the serological assay, the *Toxoplasma*-specific IgM antibody was not found in any of the patients with AD and the control group. It seems that most APs were infected, before developing the AD. In the present study, there was no significant association between AD and toxoplasmosis ( $P = 0.99$ ), however, there was a statistically significant association between the variables of age, gender, place of residence, type of nutrition and infection with *T. gondii* in the case and control groups (Table 3). These variables can be considered as important risk factors for AD.

The higher prevalence of *T. gondii* infection in APs compared to controls also showed the possible impact of this parasite in an outbreak of AD and exacerbation of symptoms, which requires special attention of specialist medi-



**Table 4.** Influence of Age, Gender, Type of Drinking Water and Serology Test Result on Alzheimer's Disease Using Multivariate Logistic Regression

Variable	Odds Ratio (OR)	Confidence Interval (CI, 95%)	P value
Age	1.19	(1.12-1.25)	< 0.001
<b>Gender</b>			
Male	Reference	-	-
Female	3.2	(1.07-9.49)	0.036
<b>Type of drinking water</b>			
Well	Reference	-	-
Piping	0.47	(0.07-2.97)	0.422
<b>Serology test result</b>			
Negative	Reference	-	-
Positive	0.99	(0.32-3.08)	0.99

cal doctors and patients encourage. The present study was the first survey in which the seroepidemiology of *T. gondii* infection was investigated in APs in Iran. One limitation of our study was the lack of use of molecular techniques such as PCR and comparison with the ELISA test. In the event, it was possible to achieve more accurate and more detailed results.

#### Footnote

**Conflict of Interest:** None of the authors had any conflict of interest concerning the financial, material, or methods used in this study.

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