# Memory impairment and latent toxoplasmosis; association, awareness and risk factors in a high Toxoplasma-seroprevalence community

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| Article Info | ABSTRACT |
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| **Article type:**<br>**Research Article**<br><br>**Received:**<br>**7 January 2021**<br>**Revised:**<br>**24 February 2021**<br>**Accepted:**<br>**20 March 2021** | **Background and Objective:** Memory impairment (MI), happens when a person loses the ability to remember information and events they would normally be able to recall. Toxoplasmosis is one of the most important parasitic diseases involving the brain. Due to the localization of Toxoplasma gondii tissue cysts in brain and some related reports, the effect of toxoplasma on neurodegenerative diseases has been suggested.<br>**Methods:** The present study was conducted to investigate the probable association between MI and toxoplasmosis using serological and molecular techniques. The study population consisted of 87 Alzheimer's patients (AP) and 87 healthy controls which were selected under the supervision of neurology consultant. The Toxoplasma-specific antibodies were measured using commercial ELISA kits. The desired region for Toxoplasma B1 gene was amplified by using specific primers and a thermocycler. Specificity of primers was confirmed by direct sequencing, aligning and phylogenetic analysis of the amplicons.<br>**Findings:** Prevalence of toxoplasmosis in AP and control group was 66.6% and 56.3% (P=0.99) and 52.8% and 40.2% (P=0.229) using ELISA and PCR respectively. Despite the higher prevalence of toxoplasmosis in AP compared with the controls, a significant relationship was not found between MI and toxoplasmosis. The multiple sequence alignment of T. gondii isolates revealed a common haplotype. The significant relationship between some variables and toxoplasmosis as well as the MI could reveal the risk factors for MI.<br>**Conclusion:** These results provide fresh insights into the ambiguous association between T. gondii infection and MI. As a probable or concomitant risk factor, toxoplasmosis could induce the MI, principally in patients with the chronic or latent infection.<br>**Keywords:** Memory Impairment, Probable link, Seromolecular, Toxoplasmosis |

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## Introduction

Alzheimer's disease is the most common form of dementia, a general term for memory impairment (MI) and other intellectual abilities serious enough to interfere with daily life which has risen in prevalence to an estimated 20-30 million people worldwide [1,2]. MI or amnesia, happens when a person loses the ability to remember information and events they would normally be able to recall. The memory loss accounts for 60 to 80 percent of dementia cases [2,3]. MI, is defined as a debilitating neurodegenerative disorder characterized by the progressive loss of cholinergic neurons, leading to the onset of severe behavioral, cognitive and motor impairments [4]. It is estimated that 30–50% of patients with Alzheimer's disease have comorbid depression [5]. Various mediators releasing from microglial cells such as cytokines, reactive oxygen species, complement factors, neurotoxic secretary products, and free radicals also augment inflammatory responses and many of these mediators are known to stimulate amyloid precursor protein deposition and contribute to neuronal death in MI [4,5]. One of the early symptoms of MI is the gradual impaired memory, especially spatial memory in which reducing the amount of acetylcholine as a result of releasing more acetyl cholinesterase enzyme can disrupt spatial memory5. Different studies have shown that transfer of oxygen to the brain and decreased blood flow may act as a mechanism in the complex etiology of Alzheimer's disease [3,5,6].

Toxoplasma gondii is a neurotropic obligate intracellular protozoan that causes the toxoplasmosis, one of the most important parasitic diseases involving the brain. Found worldwide, T. gondii is capable of infecting virtually all warm-blooded animals [7,8]. In areas where people consume raw or undercooked meat containing the tissue cyst of parasite and cats as the definitive host, live close to human, the rate of infection is higher [9]. Different reports showed that chronic Toxoplasma infection may alter the human behavior and affect the memory10,11. Behavioral changes and/or memory impairment attributed to Toxoplasma infection may have been due to the nerve damage caused by parasitic infection [6,10]. Biosynthesis of dopamine and/or serotonin can be directly affected by the aromatic amino acid, hydroxylases in T. gondii genome. The missing link between toxoplasmosis and mental disorders can be associated with increasing of dopamine in the brains of infected patients11. Serology testing of the T. gondii-specific antibodies is the mainly limited method to assess the possible relationship between neurodegenerative diseases and T. gondii infection. Therefore, the present study was conducted to evaluate the probable association between MI and T. gondii infection in a population with high Toxoplasma seroprevalence by using both the serology (ELISA) and molecular (PCR) techniques.

## Methods

### Study population and clinical samples

The study population consisted of 174 Alzheimer's patients (AP) and healthy controls (87 people in each group) that were selected under the supervision of a neurologist during a six-month period. The inclusion and the exclusion criteria were according to the mental status of APs and level of MI (mild, moderate, and advanced) which were determined by neurology consultant based on the associated factors such as navigation, remind, and construction. According to the above-mentioned criteria, evidence and medical records, the control group should have no neuropsychological problems. As much as possible, it was tried that the two case and group control groups be matched in terms of the residency, age and sex variables. At first, all participants signed a satisfaction form and then completed a standard clinical questionnaire

containing information about symptoms of MI and demographic characteristics. Whole blood samples were taken from each participant with and without EDTA and serum and buffy coat specimens were separated and stored at −20 °C until use.

### Serology assessment

The anti-T. gondii specific IgG and IgM antibodies were measured by enzyme-linked immunosorbent assay (ELISA) test using commercial kits (Pishtazteb Co, Iran) based on the manufacturer instructions. In the case of borderline test results, an additional sample was taken 7 days later and retested in parallel with the first patient sample.

### Molecular evaluation

PCR assay was performed in duplicate using the primer pair targeting the 35-fold-repetitive B1 gene of *T. gondii*[12]. The PCR reaction was performed in a final volume of 20 μl using the *Taq DNA Pol* 2.0 × master mix (Cinna Gen, Iran) contained 10 mM Tris–HCl, pH 8.3 (at 25 °C), 50 mM KCl, 1.5 mM MgCl$_2$, 5 μM each primer (Bioneer, Korea), 250 μM each dNTP, 0.1 U *Taq* DNA polymerase and 100 ng/μL (l μl) of extracted DNA. Reaction was conducted in a Thermal Cycler (BIO RML, USA) with an initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 20 s, annealing at 47 °C for 20 s and finally an extension step at 72 °C for 20 s followed by a 5 min final extension at 72 °C. A PCR negative-control sample omitted template DNA, which was replaced by sterile water and a positive-control sample that used extracted DNA from *T. gondii* tachyzoites RH-strain. The PCR products were electrophoresed in a 2% TAE (Tris-acetate-EDTA) agarose gel and stained with the ethidium bromide solution (1 μg/ML). The PCR amplification is expected to yield a 194 bp product for B1 positive reaction. To confirm the specificity of primers for the *T. gondii* detection, the purified PCR product was sequenced. The MegAlign program from Laser Gene Bio Computing Software Package (DNASTAR, Madison, WI) was used to construct the distance matrix among isolates sequenced and the other reported isolates. The phylogenetic tree was constructed based on maximum likelihood algorithm with kimura 2-parameter model inferred by MEGA5.05 software to demonstrate the status of analyzed sequences. The 1000 bootstrap re-sampling was conducted to evaluate the accuracy of phylogenetic tree.

### Statistical analysis

The data was analyzed using SPSS 18 software (SPSS, Chicago, IL, USA) and the Multivariate logistic regression, Chi-square and Fisher's exact tests. The odds ratios (OR) and 95% confidence intervals (CI) after adjustments were used to assessment the associations. For differences, a *P value* of < 0.05 was considered statistically significant.

## Results

### Demographic characteristics and serology status

Demographic characteristics of the participants in terms of MI are shown in Table 1. Various titers of *Toxoplasma*-IgG antibody which shows the chronic phase of toxoplasma infection, were found in 107 people. In other words, the seroprevalence of *T. gondii* IgG antibody in patients with Alzheimer's disease and the control group was obtained 66/6% (58/87) and 56/32% (49/87) respectively. The *Toxoplasma*-IgM antibody that related to acute phase of the disease, was not found in the subjects. The rate of *T. gondii* infection in piped water consumers, with a mixed diet, residents of the city, older than 60 years, and

illiterates was significantly higher than other groups (*P<0.05*) (Table 1). According to Chi-square and Multivariate logistic regression tests, with all other variables assumed constant, there was not a significant relationship between results of serology test and MI (*P=0.926*) (Table 2). In the present study in terms of stages of MI, 17.2%, 12.6% and 70.1% of patients were in the mild, moderate and advanced stages of MI respectively.

| Variable | ELISA | | | PCR | | |
|---|---|---|---|---|---|---|
| | Positive N (%) | Negative N (%) | *P* value | Positive N (%) | Negative N (%) | *P* value |
| **Age** | | | | | | |
| (Mean ± SD) | 65 ± 20.3 | 56.92 ± 23.1 | 0.016 | 64 ± 22.3 | 60 ± 21.12 | 0.22 |
| **Sex** | | | | | | |
| Male | 38 (21.8) | 28 (16.1) | 0.43 | 35 (20.1) | 31 (17.8) | 0.21 |
| Female | 69 (39.7) | 39 (22.4) | | 46 (26.4) | 62 (35.6) | |
| **Residency** | | | | | | |
| City | 54 (31.0) | 57 (32.8) | < 0.001 | 23 (13.2) | 88 (50.6) | <0.001 |
| Village | 53 (30.5) | 10 (5.7) | | 58 (33.3) | 5 (2.9) | |
| **Level of education** | | | | | | |
| Illiterate | 64 (36.8) | 30 (17.2) | | 50 (28.7) | 44 (25.3) | |
| Elementary | 27 (15.5) | 12 (6.9) | | 14 (8.0) | 25 (14.4) | |
| Cycle | 6 (3.4) | 6 (3.4) | 0.005 | 9 (11.1) | 3 (1.7) | 0.037 |
| Diploma | 8 (4.6) | 9 (5.2) | | 8 (9.9) | 9 (5.2) | |
| Advanced Diploma | 0 (0.0) | 2 (1.1) | | 0 (0.0) | 2 (1.1) | |
| Bachelor | 2 (1.1) | 8 (4.6) | | 0 (0.0) | 10 (5.7) | |
| **Diet type** | | | | | | |
| Most vegetables | 0 (0.0) | 0 (0.0) | | 12 (6.9) | 2 (1.1) | |
| Most protein | 13 (7.5) | 1 (0.6) | 0.010 | 69 (39.7) | 91 (52.3) | 0.004 |
| A mixture of both | 94 (54.0) | 66 (37.9) | | 0 (0.0) | 0 (0.0) | |
| **Type of water consumed** | | | | | | |
| Piping | 93 (53.4) | 67 (38.5) | 0.001 | 67 (38.5) | 93 (53.5) | <0.001 |
| Well | 14 (8.0) | 0 (0.0) | | 14 (8.0) | 0 (0.0) | |

**Table 1.** Sociodemographic variables of participants in the study based on infection with T. gondii.

### *Molecular evaluation*

The results of PCR assay indicated that 46 (52.8%) of AP and 35 (40.2%) of control subjects are positive for the *T. gondii* DNA (Fig. 1). Based on analysis of the data by Chi-square and Multivariate logistic regression tests with all other variables assumed constant there was not a significant relationship between

the results of PCR assay and MI (*P=0.226*) (Table 2). While, there was a significant relationship between the results of PCR test and diet type (*P=0.004*) and residency (*P<0.001*) according to data analysis by *Chi-Square* tests (Table 1).

According to the multiple sequence alignment of the purified PCR products it was found that the obtained partial sequences were similar to the corresponding sequences of B1 gene reported in GenBank (common haplotype; Accession No., AF179871), which proved that the PCR primers of the B1 genomic target are highly specific for the detection of *T. gondii*. The current identified *T. gondii* isolates (Park1*-Park 4*) had a percent identity (93.6-100%) and divergence (0-6.7%) with other sequences from Slovakia, Brazil, India and Southwest of Iran. The status and topology of identified T. gondii isolates with bootstrap values higher than 70% were supported in their specific complex by conducting Phylogenetic analyses.

| Variable | Group | Odds ratio (OR) | Confidence interval (CI, 95 %) | *P*. value |
|---|---|---|---|---|
| **Age** | - | 1.2 | (1.13-1.27) | 0.001< |
| **Sex** | Male | Reference | | - |
| | Female | 3.41 | (1.13-10.3) | 0.036 |
| **Type of water consumed** | Well | Reference | - | - |
| | Piping | 1.41 | (0.19-10.2) | 0.734 |
| **Result of serology test** | Negative | Reference | - | - |
| | Positive | 0.92 | (0.30-2.85) | 0.926 |
| **Result of molecular test** | Negative | Reference | - | - |

**Table 2**. Influence of age, sex, type of water consumed and result of serology and molecular tests on memory impairment by multivariate logistic regression.

## 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, transplacental transmission, blood transfusion and organ transplantation7,12. Throughout the acute stage of disease tachyzoites, the rapidly divided form of parasite disseminated via the lymphatic system or blood to different organs, causing toxoplasmosis, which is characterized by hyperplasia of the reticular cells and lymphadenopathy. However, the brain is the most commonly affected organ during the chronic, latent infection of toxoplasmosis [7,13]. In this regard, different epidemiological evidences indicate the probability of an association between exposures to T. gondii and increased the risk of neurodegenerative

disorders. Additionally, the genetics and neurobiology studies of T. gondii unraveled the evolutionary mechanisms by which parasite replication within the brain can alter behavior in mammals, thus providing a theoretical framework for the relationship between toxoplasmosis and neurological disease in humans [11,14,15].

In the present study, serological and molecular assessments of the participants revealed the higher prevalence of T. gondii infection in AP compared to the control group. In a study conducted by Kusbeci et al. [16] patients with Alzheimer showed significantly higher titers in seropositivity than healthy controls (44.1% versus 24.3%). The possible association between Toxoplasma infection and Parkinson disease (PD) was investigated by Miman and colleagues15 by evaluating the anti-T. gondii IgG antibodies. The seroprevalence rate of toxoplasmosis in patients with PD and control groups were 42.3% and 22.5%, respectively, and they were statistically significant (P=0.006). In another case-control study, the presence of T. gondii IgG and IgM antibodies were examined in 50 schizophrenic patients and 150 control subjects using ELISA, in which both the seroprevalence and the level of T. gondii IgG antibodies were higher in schizophrenic patients (20%) than in control subjects (5.3%) (P=0.003) [17]. Likewise, in the two other studies, seropositivity rate for anti-T. gondii IgG antibodies was found to be significantly more in patients with obsessive-compulsive disorder (OCD) and PD compared to the control groups [15]. Consequently, the authors concluded that toxoplasmosis may be involved in the complex mechanisms of psychiatric illness and neurodegenerative disorders. However, the anti-T. gondii IgG titers showed IgG levels examined at the same time, and thus, do not indicate a causal relationship between T. gondii infection and the etiology of neurodegenerative disorders. Since the causal relationship between T. gondii infection and the etiology of psychiatric disorders can be determined by conducting more sensitive and specific tests under known conditions along with information on times between disease onset and infection [18,19].

Assuming constant consideration of other variables, based on the Chi-Square test, the probability of developing Alzheimer's disease in people with toxoplasmosis was almost twice as high as that of healthy subjects, which the relationship was not statistically significant. Despite the indirect relationship, the result suggests that toxoplasma infection can be considered as an important risk factor for MI. With of all other variables assumed constant, for each unit increase in the age, the chance of developing MI increased 19% and 20% by serological and molecular assays respectively that this increases were significant (Table 2). El-Sahn and colleagues18 reported that the Toxoplasma infection increased with age in both schizophrenia and healthy control groups and there isn't a significant association with sex. In the present study, there was a significant relationship between age, sex, location and types of food variables with the Toxoplasma infection among the two cases and control groups which can be considered as risk factors for MI. In this study, there was a statistically significant association between the symptoms of mood change (anger), incontinence and forgetfulness with the results of serology and molecular tests that represent the possible relationship between behavioral changes with T. gondii infection. In terms of the impact of toxoplasmosis on personality, infected men seemed more jealous, less confident, more dogmatic, less impulsive, more orderly and more cautious than others. Conversely, infected women appeared to be warmest, more insecure, more sanctimonious, more persistent and more conscientious than others which these behavioral differences between Toxoplasma-infected and healthy subjects may be due to differences in the level of testosterone [11]. In the current study, no new haplotype was found among the analyzed sequences of Toxoplasma B1 gene. The small effective sample size, short length of the amplified B1 gene sequence (194 bp) and to be conserved the nature of B1 gene may be effective in this issue. The low number of patients, a wide age range of participants and lack of cooperation of all patients, were the limitations of the study.

## Conclusion

The results of the study provide fresh insights into the ambiguous association between T. gondii infection and MI. As a probable or concomitant risk factor, toxoplasmosis could induce the MI, principally in patients with the chronic or latent infection.

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