Seroprevalence and Serointensity of Latent *Toxoplasma gondii* in a Sample of Elderly Adults With and Without Alzheimer Disease

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Introduction: Latent infection with *Toxoplasma gondii* has been associated with behavioral and cognitive changes in animal models and in humans. Early findings have suggested an association between latent toxoplasmosis and Alzheimer disease (AD). On the basis of these factors, we sought to determine whether there is an association between latent toxoplasmosis and AD using a large, well-characterized sample of subjects with AD and age-matched and sex-matched controls without dementia.

Methods: Using ELISA, we determined anti-*T. gondii* IgG antibody titers in 114 control subjects and in 105 subjects diagnosed with AD through an Alzheimer's Disease Research Center.

Results: There were no group differences between groups in age, ethnicity, or sex. Education and socioeconomic status was slightly higher in the control group. Neither the prevalence of anti-*T. gondii* IgG antibodies (33% in the nondemented control group compared with 41% in the AD group, $P = 0.25$) nor log-transformed antibody concentration (106.6 IU/mL in the control group compared with 140.9 IU/mL in the AD group, $P = 0.85$) differed between the control and AD groups.

Discussion: In this sample, we found neither a higher prevalence of latent toxoplasmosis in the AD group compared with the control group nor differences in serum anti-*T. gondii* IgG titers between groups.

Key Words: Alzheimer disease, *Toxoplasma gondii*, toxoplasmosis, neurodegeneration

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The most common type of dementia and the sixth leading cause of death in the United States, Alzheimer disease (AD) causes progressive brain deterioration characterized by marked cognitive decline (Alzheimer's Association, 2013). Factors associated with the development of AD include accumulation of β-amyloid and tau proteins, genetic variants such as in the presenilin and ApoE genes, family history, and cardiovascular risk factors.[1–3]

Infectious diseases also have been implicated in the pathogenesis of AD, in part based on associations between various infectious diseases and cognitive dysfunction. In

this regard, Katan et al[4] found an association between a history of exposure to bacterial and viral pathogens and cognitive function. Similarly, other studies have shown an association between the protozoal parasite *Toxoplasma gondii* and cognitive, behavioral, and neurological changes in humans and animal models, suggesting that *T. gondii* may be a possible factor in the development of cognitive decline and AD.[5,6]

T. gondii has high affinity for the central nervous system in intermediate hosts, forming cysts in brain and muscle tissue, including neurons and astrocytes, where it can remain for the life of the host.[5–7] The worldwide prevalence of latent toxoplasmosis in humans is estimated to be between 30% and 50%, but estimates vary significantly by country and have been reported to be as high as 84%.[8] The prevalence of latent toxoplasmosis appears to increase with age.[9] To date, a single study has suggested an association between latent toxoplasmosis and AD. In this study, Kusbeci et al[10] studied a sample of 71 subjects and found a significantly higher prevalence of latent toxoplasmosis in AD (n = 34) compared with nondemented controls (44.1% and 24.3%, respectively, $P = 0.005$).

On the basis of the associations between latent toxoplasmosis and cognitive impairment and the preliminary evidence suggesting that latent toxoplasmosis could be associated with AD, we sought to investigate the seroprevalence and serointensity of latent toxoplasmosis in a comparatively large and well-characterized sample of AD subjects and age-matched controls without dementia, hypothesizing that the seroprevalence of toxoplasmosis would be higher in AD subjects than in the control group.

METHODS

Subjects

We obtained deidentified serum samples from 219 subjects from the Alzheimer's Disease Research Center (ADRC) at Washington University in St Louis, MO. A total of 105 subjects were diagnosed with AD, and 114 were identified as age-matched controls without dementia. Demographic information is presented in Table 1. Determination of AD diagnosis at Washington University was made by their clinical core (eg, neurology) based on standard guidelines.[11,12] In our analysis, we excluded subjects with a diagnosis of mild cognitive impairment from both groups. Furthermore, with a few exceptions, all subjects with a diagnosis of AD in our study had a clinical dementia rating[13] score of 2 (moderate) or 3 (severe), and with a few exceptions, all control subjects had a clinical

TABLE 1. Characteristics of the AD and Control Group

Characteristics	AD	Controls	*t* (CI), *P*
Sample size	105	114	
Age [mean, SD (range)] (y)	80.4, 7.2 (61.6-97.8)	79.7, 7.3 (62.1-98.3)	−0.7 (−2.6, 1.3), 0.49
Women (%)	54	49	−0.8 (−0.2, 0.1), 0.45
Ethnic background (white)	100	100	N/A
Education (mean, SD) (y)	13.9, 3.4	15.0, 3.0	2.5 (0.2, 1.9), 0.01
SES (Hollingshead Index) (mean, SD)	3.4, 1.2	3.8, 1.0	2.7 (0.1, 0.7), 0.01
Anti-*T. gondii* IgG Ab concentration [mean, SD (range)] (IU/mL)	140.9, 260.7 (0-1351.8)	106.6, 193.0 (0.3-940.8)	−1.1 (−95.1, 26.5), 0.27

AD indicates Alzheimer disease; CI, confidence interval.

dementia rating score of 0 (no dementia). Cognitive status of all subjects was consistent with their diagnosis of either AD or control. Socioeconomic status was estimated with the Hollingshead 2-factor index of social position.[14] In addition, the frequency of a history of hypertension, diabetes, heart disease, thyroid disease, alcohol abuse, tobacco use, and hypercholesterolemia was not significantly different between the 2 groups, although a history of stroke was significantly more prevalent in the AD group than in the control group (*P* = 0.03) (4.7% vs. 0.9%).

Procedure

Serum samples were assessed by quantitative immunoenzymatic determination of *T. gondii* IgG-class antibodies using micro-enzyme-linked immunosorbent assay (ELISA) analysis with a commercially available kit. All assays were performed according to the protocol provided by the manufacturer (GenWay; BioTech) and all samples were run in duplicate. Samples were considered positive if the IgG anti-*Toxoplasma* titer was determined to be >35 IU/mL. Those samples that measured <30 IU/mL were considered nonreactive. All samples testing in the equivocal range (30 to 35 IU/mL) (2 samples overall) were rerun. In addition to the standards provided in the kit, a known positive sample was added when assaying each plate as an additional control. Samples with absorbencies outside the standard curve were diluted 5- and 10-fold and reassayed with absorbencies adjusted accordingly by dilution factor. Lastly, 3 to 4 patient samples from each plate were rerun a second time as a quality control measure to ensure reproducibility. The person conducting the antibody assays was blind to sex and the ADRC diagnosis, and the same person performed all of the assays to reduce the influence of pipetting error on the results.

In addition to assessing for anti-*T. gondii* IgG-class antibodies, we also assayed for anti-*T. gondii* IgM antibodies in those samples that were positive or borderline positive for IgG-class antibodies. IgM antibodies are formed during an active infection and are indicative of an acute infection. A positive test for IgM antibodies specific for *T. gondii* indicates an ongoing infection, whereas a positive test for IgG antibodies indicates a past or latent *T. gondii* infection. In the IgM analysis, we again used ELISA (GenWay; BioTech) according to the protocol provided by the manufacturer. A positive and negative control was run in all experiments, and all samples were run in duplicate with the person performing the assays blind to a sample's diagnosis of AD. The *Toxoplasma* IgM index was obtained by dividing the mean values of each sample by the calibrator mean value with a *Toxoplasma* IgM index

>1 being considered positive for IgM antibodies to *T. gondii*, an index between 0.91 and 0.99 equivocal, and an index <0.90 negative. The positive and negative controls on all assays were within the index range for positive and negative controls.

Statistical Analysis

We used descriptive statistics to characterize the sample according to sex, age, seroprevalence, and average antibody titer. Because the antibody titers were not normally distributed, we used a natural-logarithm transformation to normalize the distribution. We then used *t* tests to compare latent toxoplasmosis seroprevalence between the AD and control groups and to compare the transformed anti-*T. gondii* antibody titers between the 2 groups. We also compared latent toxoplasmosis seroprevalence and serointensity between the AD and nondemented control groups in females and males separately. Finally, we used logistic regression to determine whether log-transformed anti-*T. gondii* antibody titers predicted AD while controlling for age, educational attainment, sex, and socioeconomic status. To evaluate statistical power, we performed post hoc power analyses to estimate the number of subjects required to obtain 80% power at a significance level of 0.05.

RESULTS

The final sample contained 105 subjects with AD and 114 age-matched controls without dementia. The overall prevalence of latent toxoplasmosis in the entire sample was 37.0%. The average age of the subjects diagnosed with AD was 80.4 years, and the average age of the nondemented controls was 79.7 years (*P* = 0.49), although power analysis showed that the study was underpowered to determine whether age differed between the AD and nondemented control groups. Women comprised 54% of the AD group and 49% of the nondemented control group (*P* = 0.45), but the sample size was underpowered to detect whether the prevalence of women differed between the 2 samples. The average level of education was higher by 1 year in the control group (*P* = 0.01), and socioeconomic status as estimated by the Hollingshead scale was higher in the control group compared with the AD group (*P* = 0.01; Table 1). In the overall sample, the prevalence of latent toxoplasmosis did not differ between groups (AD was 41%, compared with 33% in the controls, *t* = −1.2, 95% confidence interval, −0.2 to 0.1, *P* = 0.25; Table 2). In addition, although both females and males with AD had a higher prevalence of latent toxoplasmosis compared with

TABLE 2. The *t* Tests for Presence of Latent Toxoplasmosis Between AD and Control Groups

	AD	Control	*t* (CI), *P*
Whole group, sample size	105	114	
Prevalence (%)	41	33	−1.2 (−0.2, 0.1), 0.25
Males only, sample size	48	58	
Prevalence (%)	42	36	−0.6 (−0.2, 0.1), 0.57
Females only, sample size	57	56	
Prevalence (%)	40	30	−1.1 (−0.3, 0.1), 0.27

AD indicates Alzheimer disease; CI, confidence interval.

controls, the differences were not statistically significant: males: 42% and 36%, respectively, $t = -0.6$, 95% confidence interval, −0.2 to 0.1, $P = 0.57$; females: 40% and 30%, respectively, $t = -1.1$, 95% confidence interval, −0.3 to 0.1, $P = 0.27$. There was not a statistically significant difference in natural log transformed anti-*T. gondii* antibody concentrations between the 2 groups ($t = -0.21$, $P = 0.85$; Table 3) in either the overall sample or when analyzed separately by sex (Table 3). However, post hoc power analyses consistently showed that number of subjects was too low to identify the estimates of group differences in antibody concentration or seroprevalence between groups as statistically different. In the logistic regression models, log-transformed anti-*T. gondii* antibody titer did not predict AD (odds ratio = 1.0, $P = 0.59$, 95% confidence interval, 0.8-1.1) while controlling for age, educational attainment, sex, and socioeconomic status. Similarly, we found no significant interactions between log-transformed anti-*T. gondii* antibody titer and age ($P = 0.91$), education ($P = 0.39$), sex ($P = 0.85$, and socioeconomic status ($P = 0.54$). Finally, only 2 subjects—1 from each group—were positive for anti-*T. gondii* IgM-class antibodies.

DISCUSSION

In contrast to the study by Kusbeci et al[10] and findings showing an association between latent toxoplasmosis and cognitive function, we did not find a significantly higher seroprevalence of latent toxoplasmosis determined by anti-*T. gondii* antibodies in an AD sample compared with a group of age-matched control subjects without dementia, regardless of whether we analyzed the entire sample or men and women separately. Moreover, we did not find differences in the average log-transformed anti-*T. gondii* antibody titers between the 2 groups when analyzing the whole sample and men and women separately. Further, anti-*T. gondii* antibody titers did not predict AD in the overall sample. Although the proportion of our AD sample that was seropositive for latent toxoplasmosis was comparable with what Kusbeci et al[10] reported (41% in our study compared with 44%), the difference in latent toxoplasmosis seroprevalence between the AD and nondemented control groups in this study was not statistically significant.

Several factors may account for the differences in findings between those Kusbeci et al[10] reported and our findings. Patients in our sample were older. The average age in the Kusbeci study was 68 and 63 years for the AD and control groups, respectively, whereas in our study the respective ages were 80.4 and 79.7 years. Our AD and nondemented control groups were not completely matched in education and socioeconomic status. The differences between groups were small, however, and it is unclear how and if such a difference would affect our results. The control group in our study had higher average educational attainment and socioeconomic status than did the AD group. The Kusbeci et al[10] study did not provide detailed information regarding education, ethnicity, or socioeconomic status, other than reporting that there were no differences between groups and that seroprevalence was not correlated with these variables. Prior research on the association between latent toxoplasmosis and cognition, including seroprevalence, in young to middle-aged adults without dementia has demonstrated significant interactions between latent toxoplasmosis and lower socioeconomic status, lower education, and ethnicity.[15] As such, it is possible that an association between AD and seropositivity for toxoplasmosis in our study may have been attenuated in some manner. For instance, as our sample of subjects was clearly older than the Kusbeci et al[10] sample, it is possible that subjects seropositive for latent toxoplasmosis may have died at a higher rate than those seronegative for latent toxoplasmosis. The higher educational attainment and socioeconomic status in our control group could have protected against adverse effects of latent toxoplasmosis, masking an association between AD and latent toxoplasmosis. Because our sample was white, we could not investigate possible interactions between latent toxoplasmosis and ethnicity on AD.

Another possible reason for not finding an association between latent toxoplasmosis and AD is the difference in virulence in human diseases between different strains of *T. gondii*, as different strains are associated with differences in virulence.[16] We did not identify specific strains of *T. gondii* in our sample and, accordingly, could neither control for strain difference in our sample nor compare the strain composition between our sample and that of Kusbeci

TABLE 3. The *t* Tests for Seropositivity of Latent Toxoplasmosis Between AD and Control Groups With Natural Log-transformed Data

	AD	Control	*t* (CI), *P*
Whole group, sample size	105	114	
Anti-*T. gondii* IgG Ab [mean, SD (range)] (IU/mL)	140.9, 260.7 (0-1351.8)*	106.6, 193.0 (0.3-940.8)*	−0.2 (−0.5, 0.4), 0.85
Males only, sample size	48	58	
Anti-*T. gondii* IgG Ab [mean, SD (range)] (IU/mL)	175.2, 328.2 (0-1351.8)*	105.7, 175.6 (0.3-816.5)*	0.1 (−0.6, 0.7), 0.95
Females only, sample size	57	56	
Anti-*T. gondii* IgG Ab [mean, SD (range)] (IU/mL)	112.1, 184.4 (0-779.8)*	107.6, 211.1 (0.8-940.8)*	−0.4 (−0.7, 0.5), 0.71

*Nontransformed data—original values.
AD indicates Alzheimer disease; CI, confidence interval.

et al.[10] Similarly, host factors likely affect the virulence of *T. gondii*.[16] We did not evaluate genetic or other host factors in our sample that could affect the virulence of latent toxoplasmosis on human disease.

The results of the IgM analysis—only 1 subject in each group was positive for anti-*T. gondii* antibodies—indicate that the subjects in our sample who were positive for anti-*T. gondii* antibodies had latent but not acute toxoplasmosis. The low prevalence of anti-*T. gondii* IgM antibodies also suggests that in older adults, AD does not reactivate latent toxoplasmosis, making it unlikely that any association between latent toxoplasmosis and AD is due to reactivated toxoplasmosis.

Several additional limitations should be considered in the interpretation of our findings. The cross-sectional design we used precludes us from evaluating survivor effects. That is, subjects with AD seropositive for latent toxoplasmosis may have differentially died before being assessed for latent toxoplasmosis, equalizing the seroprevalence of latent toxoplasmosis between the 2 groups. Our study is the largest to date investigating the association between AD and latent toxoplasmosis, but the subject number was still comparatively low, and the study was underpowered to identify differences in the seroprevalence of latent toxoplasmosis between groups as statistically significant. Furthermore, the demographic characteristics of our sample limit the generalizability of these findings to other groups. Despite our findings of no differences in latent toxoplasmosis seroprevalence or antibody concentration between the AD group and age-matched controls without dementia, further research is indicated exploring the association between latent toxoplasmosis and AD in other larger samples taking into account different strains of *T. gondii*. Given the widespread distribution of latent toxoplasmosis and the need to identify potentially modifiable risk factors for AD, any association between latent toxoplasmosis and neurodegeneration requires careful investigation.

In conclusion, despite 1 previous report showing a possible association between latent toxoplasmosis and AD, we found no evidence of an association between latent toxoplasmosis and AD in a cross-sectional study. Limitations associated with this study, however, indicate that additional work investigating an association between latent toxoplasmosis and AD and other neurodegenerative diseases is warranted.

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