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Research Article

S100B and latent toxoplasmosis in Alzheimer's disease

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Abstract

Objectives: Alzheimer's disease (AD) is a fatal, multifactorial neurodegenerative disorder characterized by progressive neuronal loss and the loss of cognitive function. The etiology has not yet been fully elucidated. It has been suggested in some studies that central nervous system infections may play a role in the development of Alzheimer's disease. The aim of the present study was to investigate a possible relationship with a latent Toxoplasma gondii (T. gondii) infection in astrocytes and S100 proteins released as a result of astrocyte damage.

Methods: A total of 33 patients with AD and 32 healthy individuals were included in this study. There were 16 Toxoplasma-negative and 17 Toxoplasma-positive patients in the AD group, and 15 Toxoplasma-negative and 17 Toxoplasma-positive individuals in the control group.

Results: There were no statistically significant differences between the groups in terms of mean age or gender. An inter-group comparison of the subjects revealed that the S100B level was higher in patients with AD than in the control group ($p<0.05$). There was no statistically significant difference between the groups in terms of the T. gondii immunoglobulin G test ($p>0.05$).

Conclusion: In our study, although there was no relationship between T. gondii infection and AD, significantly higher levels of S100B in patients with AD suggest that this protein may be important both in diagnosis and in possible treatment processes. The authors suggest that reproduction of the current study using different genotypes of T. gondii would further contribute to knowledge of the etiology of AD.

Keywords: Alzheimer's disease, S100B, toxoplasma gondii

Alzheimer's disease (AD) is a fatal neurodegenerative disorder characterized by progressive neuronal loss and loss of cognitive function. AD is the most common type of dementia and its prevalence is approximately 10% after 90 years of age [1]. The number of cases worldwide in 2005 was 47 million and it is expected to be 131 million in the year 2050 [2].

Although neuronal loss and brain dysfunction is well known in AD, its pathogenic mechanism is not fully clear. Studies in the literature have focused on 3 pathological conditions in patients with AD: amyloid plaques, neurofibrillary tangles, and astrogliosis [3-5]. The development of amyloid plaques is related to the pathologies of astrocytes [6], and this condition may be induced by neurotoxic molecules, cytokine secretion, or the activation of the complement system [7].

Toxoplasma gondii is a protozoan parasite that infects both animals and humans. In humans it is usually transmitted through the ingestion of the oocyst, which can be found in cat feces, or undercooked meat containing tissue cysts [8]. Due to the high neurotrophic effects of the parasite, the most affected body tissue in a case of chronic infection is the brain [9]. This infection causes neurodegenerative changes related to neuronal inflammation in the central nervous system (CNS) [10]. Neural degeneration caused by neuroinflammation is known to be the basis of the pathogenesis in chronic neurodegenerative

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diseases, and especially in AD [11, 12]. In vitro neuropathology studies have shown that neurons, glial cells, and especially astrocytes, are selectively affected by Taxoplasma gondii (T. gondii) [13].

The S-100 protein family has been studied for 30 years. The first of this family to be discovered was S100B and it is defined as the mixture of S100A1 and S100B. The monomer of S100B is found in the CNS, especially in the cytoplasm of astrocytes [14, 15]. S100B has neurotrophic and neuroprotective effects at physiological nanomolar concentrations. However, micromolar concentrations of S100B have been shown to be neurotoxic and expressed in the process of astrocyte death [16, 17]. It was observed that in AD, the S100B level in the astrocytes was elevated in neurite plaques [18], and in another study, learning and memory impairment similar to dementia was observed in transgenic mice overexpressing S100B [19].

The aim of this study was to compare the S100 protein values in T. gondii-seronegative and T. gondii-seropositive AD patients to assess a potential relationship between S100 protein expressed by astrocytes in AD; T. gondii, which can cause latent infection in the brain; and the S100 protein released by astrocytes in AD, which can cause neuronal degeneration.

Materials and Methods

This study received approval from the Firat University Biomedical Studies Ethics Committee (number 15/04; 11/08/2015).

A total of 33 patients with AD and 32 healthy individuals with no known disease were included in this study. Patients with AD who suffered from any systematic disease, such as malignancy, coronary artery disease, hepatic failure, renal failure, etc., were excluded. The anamnesis of all of the participants was recorded, and included details of age, sex, disease duration, medicines used, etc. The patients were using medicines with cholinesterase inhibitors and/or N-methyl-D-aspartate receptor antagonists. Blood samples were collected from all of the patients between the hours of 8:00 am and 10:00 am after a fast of 8 to 10 hours. The serum S100B level in the samples was measured and analyzed using a Cobas 8000/e602 analyzer (Roche Diagnostics, GmbH, Risch-Rotkreuz, Switzerland). Venous blood samples were collected and placed in gel separator tubes to measure the serum S100B value. Samples were centrifuged at 3600 rpm for 10 minutes. The serum samples for S100B were placed in Eppendorf tubes and stored at -20°C. The lower detection limit was 0.005 µg/L, and concentrations of up to 39 µg/L could be measured without dilution. The intra- and inter-assay coefficients of variation for S100B were 1.8% and 2.4%. Blood samples were taken from all of the study participants under sterile conditions. The samples were centrifuged at 1000 rpm, and the sera samples were stored at -20°C until the serological examination was performed. A T. gondii immunoglobulin G (IgG) test (Vircell SL, Granada, Spain) was analyzed using the Triturus system (Grifols SA, Barcelona, Spain) and the enzyme-linked immunosorbent assay method. Positive and negative controls were included each run. Specimens with a cutoff index value of <9 were considered negative and those with a cutoff index value of >11 were considered positive for T. gondii IgG. If the result was between 9 and 11, the sample was retested and/or a new sample was obtained for confirmation.

The G*Power program (Heinrich-Heine-Universitat, Düsseldorf, Germany) was used to calculate the sample size for the study. According to the criteria developed by Cohen, the effect size was identified as small (d=0.2), medium (d=0.5), and large (d=0.8). The sample size was calculated using the sublimit (0.8) of the large effect size, as suggested by Cohen [20]. The minimum sample size for each group was determined to be 26. The effect size was found to be 0.61 in analyses conducted following the study.

Statistical analysis of our results was conducted using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY USA). The Kolmogorov-Smirnov test was used to determine whether data were normally distributed. For both descriptive statistical methods and comparison of quantitative data, Student's t-test was used. Comparisons between the subgroups were made using one-way analysis of variance. To compare qualitative data, a chi-square test was applied, and the Pearson correlation test was used to analyze relationships between parameters. The results were analyzed using a $p<0.05$ significance level and 95% confidence intervals.

Results

The study included 33 patients with AD, 14 female and 19 male, with ages ranging between 53 and 90 years. The control group consisted of 32 healthy volunteers, 11 female and 21 male, with ages ranging between 57 and 95 years (Table 1). There were no statistically significant differences between the groups in age or sex ($p>0.05$). The inter-group comparison of the subjects revealed that values of S100B in the AD

Table 1. The demographic characteristics and biochemical parameters of patients with Alzheimer's disease and the control group

	Control group n=32	Alzheimer's disease n=33	p
Female/male	11/21	14/19	0.612
Age (years)	75.19±8.54	76.73±8.69	0.474
Alzheimer' disease duration	-	5.33±2.5	-
T.gondii (negative/positive)	15/17	16/17	1.0
S100B (µg/L)	0.042±0.012	0.054±0.025	0.016

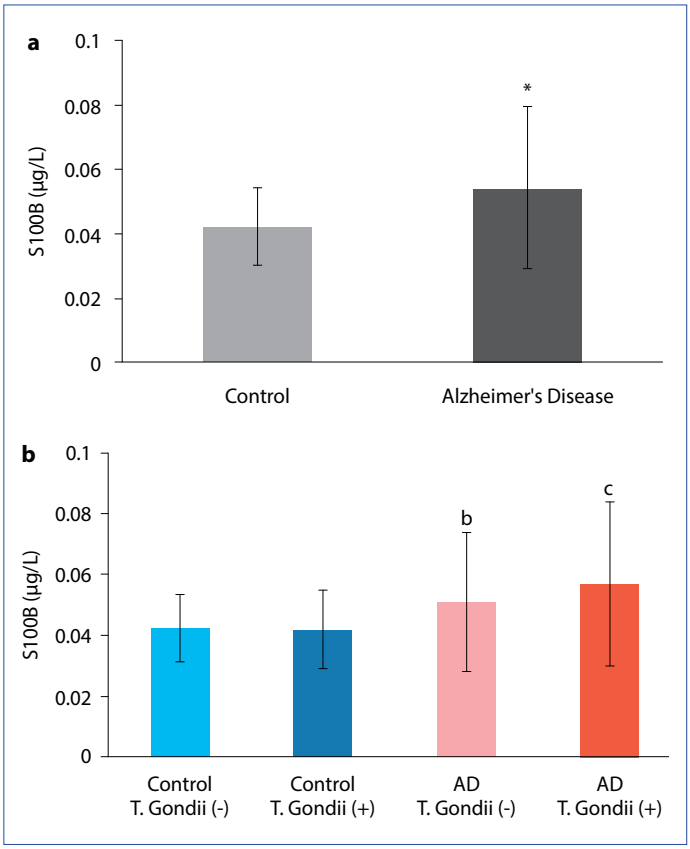

Figure 1. Comparison of S100B values of the patients and those of the control group. p<0.05 when compared with the control group.

(0.054±0.025 µg/L) were higher than those of the control group (0.042±0.012 µg/L) (Fig. 1a). The control and AD groups were divided into subgroups of T. gondii-negative (-) and T. gondii-positive (+). Comparison of the S100B level in the control T. gondii (-) (0.0422±0.011 µg/L) and control T. gondii (+) (0.0418±0.013 µg/L) groups revealed no significant differences (p>0.05). Furthermore, no statistically significant differences were detected when the S100B level in the AD T. gondii (-) (0.051±0.023 µg/L) and AD T. gondii (+) (0.057±0.027 µg/L) groups were compared (p>0.05) (Fig. 1b).

There were no statistically significant differences determined between the AD group and the control group in T. gondii IgG testing (p>0.05).

Discussion

Since the use of biological markers is very limited in deciding on the diagnosis, treatment, and the course of AD, new etiological studies and interpretations are required to contribute to our understanding of the physiopathology of the disease.

Researchers have conducted many studies regarding a relationship between T. gondii and both psychiatric and neurological diseases since the 1950s, and these studies have demonstrated that the relationship between T. gondii and behavioral changes should not be underestimated [21].

One of the most important hypotheses proposed regarding the unclear pathogenesis of AD is an excessive production of acute phase reactants, proinflammatory cytokines, and immunostimulant molecules as a result of the chronic activation of glial cells around the B amyloid plaques. One of these molecules is S100B. In Tg2576 rat models, S100B synthesized from astrocytes with arundic acid was blocked and it was observed that after the blockage, Alzheimer's-like pathologies decreased and in fact, cerebral amyloidosis increased in rats with an overexpression of S100B [22, 23]. Although studies regarding the effects of latent T. gondii infection on behavior and its role in the physiopathology of AD are limited, they are promising [24, 25]. In AD, neuroinflammatory parameters, such as microglial cells, astrocytes, and complement activators expressed by neurons, chemokines, cytokines, as well as oxygen radicals may provide insight to researchers regarding the physiopathology of the disease [26]. When it is considered that increased levels of S100B, which is expressed by damaged astrocytes, can create conditions like dementia and even cause behavioral pathologies [19], and that the S100B levels of Alzheimer's patients in this study were significantly higher than some reports in the literature [27], it may be that this protein is important in both diagnosis and possible treatment processes.

In a study examining the unclear physiopathology of AD, infectious agents, and blood-brain barrier abnormalities, it was stated that an excessive release of S100B may be a marker of blood-brain barrier dysfunction and neuronal damage [28]. We suggest that it would be appropriate to evaluate infectious agents that might be thought to cause AD and proteins such as S100B that are released predominantly from glial cells (astrocytes, oligodendrocytes and Schwann cells) given that both pathologies are also observed in AD and that Toxoplasma affects neurons, glial cells, and particularly astrocytes.

There are several limitations to this study. First of all, the study population was relatively small. Second, although the study results indicated that the S100B level was elevated in patients with AD, as has been previously reported [29], it is unknown if the increase resulted from any defects in the blood-brain barrier or from adipocytes and skeletal myoblast cells [30], which are extracranial sources of S100B. This will be a useful distinction to explore in future studies.

Conclusion

Continued research related to the mechanisms through which S100B affects learning and memory impairment processes will also be very beneficial. Although there was no relationship between T. gondii infection and AD determined in the present study, we believe that reproduction of the study with other genotypes of T. gondii would be a positive contribution to the literature. Xia et al. [31] observed in the study they conducted in 2017 that different T. gondii species

demonstrate different virulence levels and cause different results in humans. Studies with a larger sample size investigating various species of T. gondii are required to extend the findings of the current study.

Conflict of interest: There is no conflict of interest.

Ethics Committee Approval: This study received approval from the Firat University Biomedical Studies Ethics Committee (number 15/04; 11/08/2015).

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