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Increase in the IgG avidity index due to herpes simplex virus type 1 reactivation and its relationship with cognitive function in amnestic mild cognitive impairment and Alzheimer's disease

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ABSTRACT

After infection with herpes simplex virus type 1 (HSV-1), latent infection persists for life in the trigeminal ganglion and reactivation results in an outbreak of cold sores around the mouth. Many previous studies have reported HSV-1 reactivation to be a risk factor for Alzheimer's disease (AD). This study enrolled subjects with AD ($n = 85$), subjects with amnestic mild cognitive impairment (aMCI; a prodromal stage of AD) ($n = 34$), and healthy controls ($n = 28$). The avidity index of anti-HSV-1 IgG antibodies—a known indicator of HSV-1 reactivation—was measured in order to clarify the relationship between HSV-1 reactivation and symptoms of cognitive function in AD.

Cognitive function in AD and aMCI were evaluated using scores from the mini-mental state examination (MMSE) and frontal assessment battery (FAB). The results showed that the subjects with aMCI, for which cerebral function is better preserved than subjects with AD, had a higher anti-HSV-1 IgG antibody avidity index than the AD subjects or healthy controls. Furthermore, the anti-HSV-1 IgG antibody avidity index was even higher in the subjects with high MMSE scores on orientation to time and three-step command subscores. We observed a negative correlation between the anti-HSV-1 IgG antibody avidity index and plasma BDNF concentration, which is an indicator of encephalitis. This suggests that HSV-1 reactivation, as observed through an increase in the anti-HSV-1 IgG avidity index, does not progress to encephalitis. These results suggest that HSV-1 reactivation occurs from the stage of aMCI, which is prodromal to AD, and can affect AD symptoms without an intermediary stage of severe encephalitis. The study demonstrates that the anti-HSV-1 IgG antibody avidity index could be a useful biomarker for the early diagnosis of aMCI as well as AD, and suggests that antiviral medication to treat HSV-1 could play a role in preventing the onset of AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by memory disturbance, visuospatial agnosia, attention deficit, and difficulties with executive functions [1,2]. AD is most powerfully explained by the amyloid cascade hypothesis, whereby beta amyloid (Aβ) is produced through proteolysis of amyloid precursor protein (APP) and aggregates in the brain, resulting in the patient progressing through a stage of mild cognitive impairment (MCI) to AD.

Herpes simplex virus type 1 (HSV-1) is thought to be a risk factor for AD, based on reports that HSV-1 has been detected in senile plaques and brains of AD patients and on the high rates of anti-HSV-1 intrathecal antibodies detected in AD patients [3–6]. HSV-1 infection usually occurs during childhood, after which the virus remains dormant in the trigeminal ganglion. Stress and fatigue can reactivate HSV-1, and cause an outbreak of cold sores around the mouth [7]. HSV-1 infection is particularly widespread in the elderly, with research suggesting that over 70% of individuals aged 50 years and above are infected with HSV-1 [8].

HSV-1 affects APP transport and distribution [9], and research has shown that HSV-1 is related to Aβ plasma concentration [10], suggesting that HSV-1 affects how APP is processed within the brain. However, other research shows that HSV-1 DNA is also detected in the brain of elderly subjects without AD [11], and that

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IgG antibodies are detected in the brain at the same levels in subjects with AD and healthy individuals [6]. Therefore, researchers have yet to reach a conclusion over the relationship between HSV-1 and AD.

Other research has attempted to clarify the relationship between HSV-1 and AD by measuring anti-HSV antibodies in the peripheral blood. One study reported no difference in serum anti-HSV-IgG antibody titers between AD subjects and healthy individuals [12], whereas another study reported higher anti-HSV-IgG antibody titers in healthy individuals than in AD subjects [13]. Hence, the assessment of serum anti-HSV IgG antibody titer has not resulted in clear conclusions being drawn. We attribute this to the difficulty of using IgG antibody titer measurements to distinguish between initial HSV infection and HSV reactivation. A report on differentiating between initial HSV infection and reactivation through the measurement of both anti-HSV-IgG antibodies and anti-HSV-IgM antibodies suggested that anti-HSV-IgM antibody positivity (i.e., HSV reactivation) was a risk factor for AD onset [14].

The findings above suggest the need to distinguish between initial HSV infection and HSV reactivation when investigating the relationship between AD and HSV by measuring anti-HSV antibody titers in the peripheral blood. Compared with initial HSV infection, HSV reactivation is characterized by an increase in high-avidity anti-HSV IgG antibodies, and an avidity index that uses the ratio of high-avidity anti-HSV IgG antibody titers of wells washed with urea buffer to anti-HSV IgG antibody titers of wells washed with non-urea buffer has been found to be a good indicator of reactivation [15,16]. Therefore, in this study we measured the anti-HSV-1 IgG antibody titer and anti-HSV-1 IgG avidity index as indicators of HSV-1 reactivation in AD patients.

In order to clarify the relationship between HSV-1 and the clinical disease stage of AD, we targeted not only AD but also amnestic mild cognitive impairment (aMCI), which is thought to be a precursor stage to AD. MCI is defined as an intermediate state between normal aging and AD, and a high proportion of the aMCI subgroup (subjects with memory disturbance) progress to AD [17–19]. Our objective was to clarify the detailed relationship between HSV-1 reactivation and cognitive function in AD patients by using scores from the mini-mental state examination (MMSE) and frontal assessment battery (FAB) to evaluate cognitive function in these patients.

HSV-1 is also known to suddenly cause severe encephalitis, although the incidence is very low [7]. Given that research has shown an increase in brain-derived neurotrophic factor (BDNF) with encephalitis [20,21], we also assessed whether severe encephalitis had occurred in the study subjects at the time of testing by measuring plasma BDNF concentrations.

2. Materials and methods

2.1. Participants

The study enrolled 85 subjects diagnosed with AD and 34 subjects with aMCI who were Japanese outpatients being treated at The Jikei University Hospital (Tokyo) or The Jikei University Kashiwa Hospital (Kashiwa city, Chiba prefecture). All subjects were diagnosed with AD or aMCI based on the US National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) criteria for the diagnosis of AD or the MCI diagnostic criteria defined by Peterson [17,22]. In this study, aMCI included both amnestic MCI-single domain and MCI-multiple domain types subjects. Subjects were excluded from the study if they had dementia with Lewy bodies (DLB), frontotemporal dementia, vascular dementia, normal pressure hydrocephalus (NPH), other CNS disease, head trauma, substance-related disorder, major depressive disorder, psychotic disorder, epilepsy, or delirium. Subjects were also excluded if they had been diagnosed with their condition for over 3 years, because the objective of the study was to evaluate early stage aMCI and AD. Cognitive function was evaluated using scores from MMSE (ranging from 0 to 30) [23] and FAB (ranging from 0 to 18) [24,25]. Whole blood samples were collected on the same day as the cognitive function tests, centrifuged to separate the plasma, and stored at −80 °C until analysis.

The study enrolled 28 healthy individuals aged 60 years or above with no memory deficit and 13 subjects with mood disorders (eight with major depressive disorder, five with bipolar disorder) as controls. Whole-blood samples were centrifuged to separate the plasma and stored at −80 °C until analysis.

This study was approved by the Ethics Committee of The Jikei University School of Medicine and Tokyo Metropolitan Institute of Medical Science. All subjects as well as their caregivers provided written informed consent.

2.2. Anti-HSV-1 antibody titer

We used an HSV-1 IgG ELISA Kit (Phoenix Pharmaceuticals, Inc.). The anti-HSV-1 antibody titer was measured according to the attached protocol. To measure the high avidity anti-HSV-1 antibody titer, we modified the attached protocol through the addition of 6 M urea to the washing solution at the washing step after the plasma reaction. A TriStar LB941-vTi Microplate Reader (Berthold Technologies) was used to measure optical density (450 nm) after the color reaction. Following the attached protocol, the anti-HSV-1 antibody titer was expressed as an HSV-1 IgG index by comparing the patient sample optical density and the cut-off calibrator optical density. A sample was defined as being positive for anti-HSV-1 IgG antibody if the HSV-1 IgG Index was above 1.0.

The anti-HSV-1 IgG avidity index was derived as follows from the anti-HSV-1 IgG antibody positive samples: Avidity index (%) = anti-HSV-1 antibody titer measured with washing including urea/anti-HSV-1 antibody titer measured with washing without urea.

2.3. Plasma BDNF concentration

Plasma BDNF concentrations were measured using the Emax ImmunoAssay System (Promega) according to the attached protocol. The plasma was diluted five-fold with Dulbecco's PBS, and the optical density (450 nm) after the color reaction was measured using a TriStar LB941-vTi Microplate Reader (Berthold Technologies).

2.4. Statistical analysis

ANOVA was used to compare most of the background characteristics of subjects in the aMCI, AD, and healthy control groups. The exceptions were gender and percentage of subjects positive for anti-HSV-1 antibodies, which were compared using the chi-squared test. The unpaired Welch's t test was used to compare age, years of education, duration of disease, MMSE and FAB scores between two groups. The Kruskal–Wallis test was used to compare anti-HSV-1 IgG antibody titers and anti-HSV-1 antibody avidity indices in the aMCI, AD, and healthy control groups. The Mann–Whitney U test was used for comparisons between two groups regarding the anti-HSV-1 IgG antibody titers and anti-HSV-1 antibody avidity indices. Spearman's rank correlation coefficient was used to investigate the correlation between variables. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS Statistics version 19 (IBM) and Prism 5 (GraphPad).

3. Results

3.1. Study subject characteristics

There were significant differences among the healthy control, aMCI, and AD groups in terms of age, gender, and years of education. Furthermore, a comparison of the aMCI and AD groups showed that the AD group had significantly lower MMSE and FAB scores. No significant differences were observed for gender, years of education, and duration of disease between the aMCI and AD groups. No differences were observed between the healthy control, aMCI, and AD groups in the percentage of subjects positive for anti-HSV-1 antibodies (Table 1).

3.2. HSV-1 antibody titer

The anti-HSV-1 antibody titer was higher in the aMCI group than in the healthy control group ($P \leqslant 0.05$, Mann–Whitney U test). No significant difference was observed in anti-HSV-1 antibody titer between the healthy control and AD groups or between the aMCI and AD groups (Fig. 1A). Furthermore, no significant difference was observed in anti-HSV-1 antibody titer between the healthy control and mood disorder groups (data not shown). The anti-HSV-1 antibody avidity index was higher in the aMCI group than in the healthy control and AD groups ($P < 0.05$, $P < 0.05$, Mann–Whitney U test) (Fig. 1B). No significant difference was observed in anti-HSV-1 antibody avidity index between the healthy control and mood disorder groups (data not shown). No correlation was observed between age and anti-HSV-1 antibody titer or between age and anti-HSV-1 antibody avidity index ($\rho = 0.13$, $P = 0.11$, $\rho = 0.08$, $P = 0.41$, Spearman's rank correlation coefficient). Furthermore, no correlation was observed between years of education and anti-HSV-1 antibody titer or between years of education and anti-HSV-1 antibody avidity index ($\rho = 0.14$, $P = 0.10$, $\rho = -0.04$, $P = 0.66$, Spearman's rank correlation coefficient). There was no difference in anti-HSV-1 antibody titer and anti-HSV-1 antibody avidity index due to gender ($P = 0.09$, $P = 0.43$, Mann–Whitney U test).

3.3. Correlation between anti-HSV-1 antibody avidity index and MMSE score

No correlation was seen between the anti-HSV-1 antibody avidity index and the MMSE score ($\rho = 0.18$, $P = 0.08$ by Spearman's rank correlation coefficient) (Fig. 2A). We investigated the correlation between the anti-HSV-1 antibody avidity index and representative subscore on the MMSE items. The results showed a positive correlation between the anti-HSV-1 antibody avidity index and the score for orientation to time as well as between the anti-HSV-1 antibody avidity index and the three-step commands score, which represent language understanding ($\rho = 0.26$, $P < 0.05$, $\rho = 0.25$, $P < 0.05$ by Spearman's rank correlation coefficient) (Fig. 2B and C). No correlations were observed with the other MMSE subtests (orientation to place: $\rho = 0.10$, $P = 0.36$; delayed recall: $\rho = 0.18$, $P = 0.08$; immediate memory: $\rho = 0.17$, $P = 0.11$; calculation: $\rho = -0.08$, $P = 0.46$ by Spearman's rank correlation coefficient). No correlation was observed between the anti-HSV-1 antibody avidity index and the FAB score ($\rho = 0.09$, $P = 0.45$ by Spearman's rank correlation coefficient).

Table 1
Study subject characteristics (mean ± SEM).

	Control ($n = 34$)	MCI ($n = 34$)	AD ($n = 85$)	P
Age	66.8 ± 1.0	74.3 ± 0.6	78.7 ± 0.7	<0.0001
Female (%)	42.9	55.9	70.6	<0.05
Male (%)	57.1	44.1	29.4	<0.0.5
Education (years)	13.7 ± 0.5	12.4 ± 0.5	11.5 ± 0.3	<0.005
Duration of disease (months)	–	16.7 ± 1.5	20.2 ± 0.9	0.05
MMSE score	–	26.9 ± 0.4	19.6 ± 0.5	<0.0001
FAB score	–	14.4 ± 0.4	11.3 ± 0.4	<0.0001
Anti-HSV-IgG positive (%)	60.7	85.3	77.6	0.07

Gender and % of subjects positive for anti-HSV-1 IgG antibodies were analyzed using a chi-squared test. Age and years of education were analyzed using an ANOVA. Other parameters were analyzed using an unpaired Welch's t test. $P < 0.05$ was considered statistically significant.

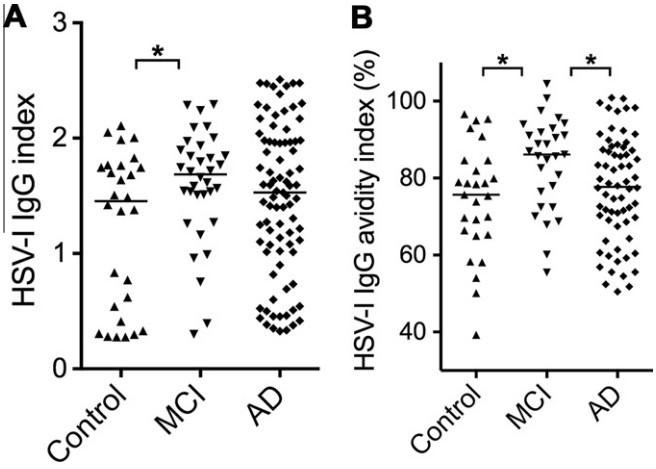

Fig. 1. Anti-HSV-1 antibody titer (A) and anti-HSV-1 antibody avidity index (B) in the healthy control, aMCI, and AD groups. Horizontal lines show the median value. *$P < 0.05$ by Mann–Whitney U test.

3.4. Correlation between anti-HSV-1 antibody avidity index and plasma BDNF

The results showed a negative correlation between the anti-HSV-1 antibody avidity index and plasma BDNF concentration ($\rho = -0.22$, $P < 0.05$ by Spearman's rank correlation coefficient) (Fig. 3).

4. Discussion

This study enrolled 28 healthy volunteers aged 60 years or older and 119 subjects who visited our clinic with memory complaints. The subjects included 34 individuals with aMCI and 85 with AD. The mean age increased in the order of healthy, aMCI, and AD subjects, which we attribute to age being a strong risk factor for AD [26,27] and to aMCI and AD being continuous disease states in which aMCI progresses to AD [17–19]. No significant differences were observed between the aMCI and AD groups for gender, years of education, or disease duration, although the MMSE and FAB scores were significantly lower in the AD group compared with the aMCI group (Table 1). Therefore, the diagnoses of aMCI and AD were considered appropriate in this study cohort.

No differences were seen in the percentage of subjects positive for anti-HSV-1 antibodies between the healthy control, aMCI, and AD groups (Table 1). The anti-HSV-1 IgG antibody titer was not significantly different between the aMCI and AD groups, but was higher in the aMCI group than in the healthy control group (Fig. 1A). Furthermore, the anti-HSV-1 IgG avidity index was higher in the aMCI group than in the healthy control and AD groups (Fig. 1B). The comparison of the aMCI and AD groups showed significant differences in age, gender, and years of education (Table 1). However, no relationship was observed between these

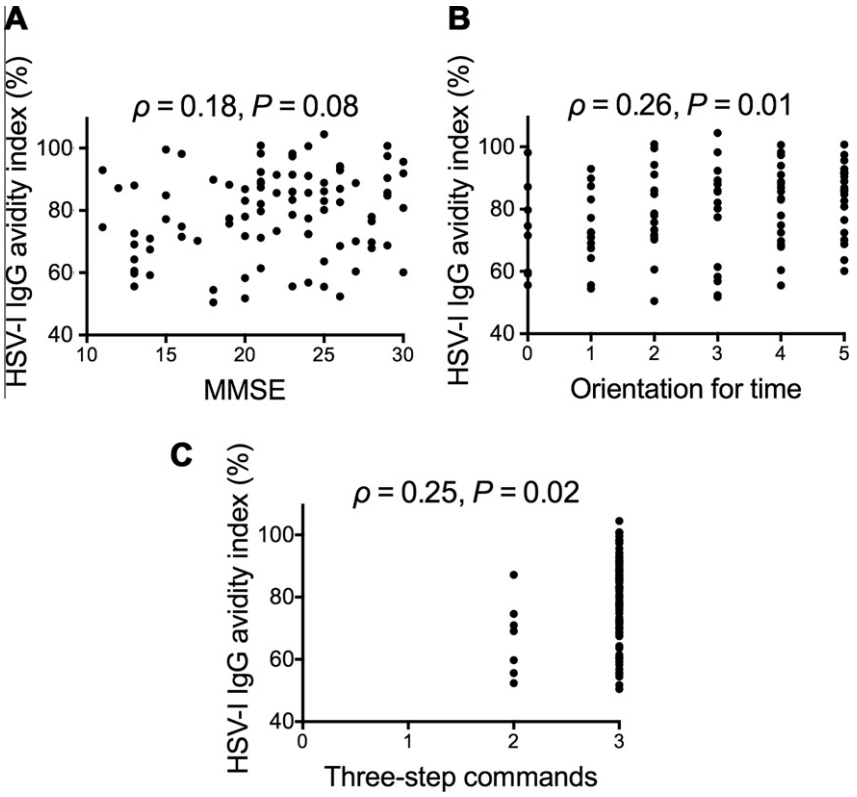

Fig. 2. Correlations between anti-HSV-1 IgG avidity index and total MMSE score (A), score for orientation to time included in the MMSE (B), and score for three-step commands that represent language understanding included in the MMSE (C). $\rho = 0.18$, $P = 0.08$ (A); $\rho = 0.26$, $P = 0.01$ (B); $\rho = 0.25$, $P = 0.02$ (C); Spearman's rank correlation coefficient.

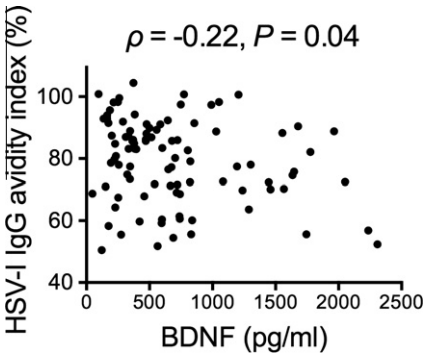

Fig. 3. Correlation between anti-HSV-1 IgG avidity index and plasma BDNF concentration. $\rho = -0.22$, $P = 0.04$, Spearman's rank correlation coefficient.

confounding factors and the anti-HSV-1 antibody titer or anti-HSV-1 IgG avidity index. Therefore, the results were negative for their involvement in HSV-1 reactivation. A higher anti-HSV-1 IgG avidity index implies that HSV-1 reactivation has occurred [15,16]. These results show that HSV-1 reactivation occurs more frequently in the aMCI group (the prodromal stage to AD) than in the healthy control and AD groups. Differences were observed between the healthy control and aMCI groups for both the anti-HSV-1 IgG avidity index and anti-HSV-1 IgG antibody titer. This strongly suggests the involvement of HSV-1 as subjects move from a healthy state to the onset of aMCI. Letenneur et al. reported that subjects were positive for anti-HSV-1 IgM antibodies before the onset of AD, indicating that HSV reactivation is a risk factor for AD onset [14]. Our results support these findings. The results suggest that HSV reactivation occurs before the onset of AD, and that HSV reactivation does not immediately damage the brain and cause cognitive impairment but must be sustained for a long period in order to cause cognitive impairment.

Although mood disorders are thought to be risk factors for AD onset [28], no HSV-1 reactivation was observed in the mood disorder subjects compared with healthy controls.

We investigated the correlation between the anti-HSV-1 IgG avidity index and MMSE scores in order to examine what sort of cognitive impairments are related to HSV-1 reactivation. The results showed a tendency towards a positive correlation between the anti-HSV-1 IgG avidity index and MMSE (Fig. 2A). In particular, we observed positive correlations with three-step commands, which represent language understanding, and with orientation to time. The results suggested that compared with subjects before the onset of AD, HSV reactivation had subsided in subjects with AD at the stage where symptoms of cognitive impairment were observed for orientation to time and language understanding. We think that this is one factor in the relationship between AD and HSV that previous research had not clarified.

In a meta-analysis on AD, Bäckman et al. concluded that cognitive impairment appears several years before a diagnosis of AD and suggested that there may be structural and functional changes in the brain some time before a confirmed diagnosis of AD [29]. This suggests that HSV reactivation could be involved in structural and functional changes in the brain before the onset of AD.

HSV-1 is known to suddenly cause severe encephalitis [7]. No clinical fever or neurological symptoms were observed in the subjects during their participation in this study. Furthermore, there was a negative correlation between the anti-HSV-1 IgG avidity index and the plasma BDNF concentration, which has been reported to increase during encephalitis [20,21]. This suggests that in aMCI patients where HSV-1 reactivation was observed, there was no

increase in plasma BDNF concentration and severe encephalitis did not occur. HSV infection has been reported to induce interleukin (IL) 1, IL-6, and tumor necrosis factor (TNF) alpha [30]. With AD, somatic infection and increased levels of inflammatory cytokines (IL-1, IL-6 and TNF-alpha) have been suggested to be involved in chronic neurodegeneration in the brain [31,32]. Therefore, even if HSV reactivation occurs outside the brain, the induction of inflammatory cytokines could be involved in the onset of AD. However, although the presence of HSV-1 within the brain does not cause severe encephalitis, it is thought to be involved in microglial activation and an increase in inflammatory cytokines, which could play a part in the onset of AD [14].

This paper shows that HSV-1 reactivation may be one risk factor for the onset of AD, which suggests that the use of antiviral medication to prevent HSV-1 reactivation could prevent AD. This research also suggests that the anti-HSV-1 IgG avidity index, an indicator for HSV-1 reactivation, could be a useful biomarker for the early diagnosis of aMCI and AD, as it can be measured easily using peripheral blood. To confirm the findings, longitudinal rather than cross-sectional research is needed, and these should be a large number of study samples.

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