CSF Biomarker Profiles in CNS Infection Associated with HSV and VZV Mimic Pattern in Alzheimer's Disease.

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Letter

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Abstract

Alzheimer's disease (AD) is the most common cause of dementia. Although AD was initially considered to be a cell autonomous neurodegenerative disorder, marked neuroinflammation is observed in the brains of patients with AD, alongside Aβ and tau pathology. Based on genetic and molecular biological findings, central nervous system (CNS) inflammatory processes have been postulated to be involved in the etiopathogenesis of AD, in which activated microglia play a key role. This has also been supported by the epidemiological observation that CNS infections were associated with the development of AD, and in particular the relationship between herpetic virus and AD has been well-investigated. For example, the presence of anti-herpes simplex virus (HSV) antibody was associated with an elevated risk of developing AD [1]. Moreover, anti-herpetic medication was associated with a reduced risk of dementia in a population-based study [2]. Similar results were also observed in the case of varicella zoster virus (VZV) infections [3]. Taking into consideration the reports above, we hypothesized that the biomarker signature representing AD might be observed in patients with herpetic viral CNS infections as a prognostic biomarker of AD development. In the current study, we aimed to determine whether or not the biomarkers related to AD and neurodegeneration were changed in patients with CNS infection by HSV and VZV compared with controls. This study focused on CSF levels of $A\beta_{1-42}$, $A\beta_{1-40}$, total-tau (t-tau), and tau phosphorylated at threonine 181 (p-tau) as molecules representing the AD signature; neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (p-NfH) as indicators of axonal injury; soluble triggering receptor expressed on myeloid cells 2 (sTREM2) as a potential biomarker for microglia activity; and glial fibrillary acidic protein (GFAP) as a biomarker for astrocytic damage. We also measured serum levels of NfL as a blood based biomarker for axonal injury. (For detailed methods, see Supplementary methods) The demographic characteristics, diagnosis, CSF profiles, results of viral detection, magnetic resonance imaging (MRI) findings, lowest score of the Glasgow coma scale (GCS) during the hospitalization period, and modified Rankin Scale (mRS) at discharge are summarized in Supplementary Table 1 and 2. There was no significant difference in age or sex among the HSV, VZV, and control groups.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia. Although AD was initially considered to be a cell autonomous neurodegenerative disorder, marked neuroinflammation is observed in the brains of patients with AD, alongside Aβ and tau pathology. Based on genetic and molecular biological findings, central nervous system (CNS) inflammatory processes have been postulated to be involved in the etiopathogenesis of AD, in which activated microglia play a key role. This has also been supported by the epidemiological observation that CNS infections were associated with the development of AD, and in particular the relationship between herpetic virus and AD has been well-investigated. For example, the presence of anti-herpes simplex virus (HSV) antibody was associated with an elevated risk of developing AD [1]. Moreover, anti-herpetic medication was associated with a reduced risk of dementia in a population-based study [2]. Similar results were also observed in the case of varicella zoster virus (VZV) infections [3]. Taking into consideration the reports above, we hypothesized that the biomarker signature

representing AD might be observed in patients with herpetic viral CNS infections as a prognostic biomarker of AD development. In the current study, we aimed to determine whether or not the biomarkers related to AD and neurodegeneration were changed in patients with CNS infection by HSV and VZV compared with controls. This study focused on CSF levels of $A\beta_{1-42}$, $A\beta_{1-40}$, total-tau (t-tau), and tau phosphorylated at threonine 181 (p-tau) as molecules representing the AD signature; neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (p-NfH) as indicators of axonal injury; soluble triggering receptor expressed on myeloid cells 2 (sTREM2) as a potential biomarker for microglia activity; and glial fibrillary acidic protein (GFAP) as a biomarker for astrocytic damage. We also measured serum levels of NfL as a blood based biomarker for axonal injury. (For detailed methods, see Supplementary methods) The demographic characteristics, diagnosis, CSF profiles, results of viral detection, magnetic resonance imaging (MRI) findings, lowest score of the Glasgow coma scale (GCS) during the hospitalization period, and modified Rankin Scale (mRS) at discharge are summarized in Supplementary Table 1 and 2. There was no significant difference in age or sex among the HSV, VZV, and control groups.

For comparison between herpetic CNS infection and control groups, we combined the data of the HSV and VSV groups, and then compared their biomarker values to those of the control (shown in Figure 1A to 1K). In this comparison, we found the following significant differences: The levels of CSF $A\beta_{1-42}$, $A\beta_{1-40}$, and the $A\beta_{1-42}/A\beta_{1-40}$ ratio were significantly lower in the HSV and VSV combined group (HSV/VSV) compared with the control group (P=0.01836, P=0.0380, and P= 0.0262, respectively) (Figure 1A, 1B, and 1C). CSF t-tau, p-tau, CSF sTREM2, and CSF GFAP levels were significantly elevated in the HSV/VSV group compared with the control group (P= 0.0043, P=0.0007, P=0.0030, and P=0.0139, respectively) (Figure 2D, 2E, 2I, and 2J). The HSV/VZV group tended to have higher CSF p-tau/t-tau, CSF NfL, CSF p-NfH and serum NfL levels compared with the control. However, these trends did not reach significance (Figure 2F, 2G, and 2H). In comparison among the HSV, VZV, and control groups (Supplementary Figure 1), elevation of CSF p-tau was marked in the VZV group while the levels of CSF t-tau were elevated specifically in the HSV group. The other biomarkers showed similar trends to those in comparison between HSV/VZV and the controls groups.

Results of uni- and multivariate regression analyses between those biomarker values and clinical severity are summarized in Supplementary Table 3. The negative correlations between GCS and NfL in CSF and serum were significant after age adjustment (P=0.014 and P=0.030, respectively) The significant correlation between CSF NfL and mRS on discharge was only preserved after age adjustment (P=0.018) among those biomarkers. (Supplementary Figure 2 showed scatter plots in cases showing significant correlations on univariate analyses)

The current study generated three major findings: First, regarding Aβ and tau- related biomarkers, the HSV/VZV group showed significantly lower CSF levels of $A\beta_{1-42}$, $A\beta_{1-40}$, and the $A\beta_{1-42}/A\beta_{1-40}$ ratio compared with the control group, while CSF t-tau and p-tau were significantly elevated in the HZV/VZV group. On comparing HSV and VZV groups, elevation of p-tau was marked in the VZV group and, while the levels of CSF t-tau were elevated specifically in the HSV group. These results correspond to previous reports showing significantly decreased Aβ1-42, increased t-tau, and increased p-tau in CSF of patients

with HSV encephalitis [4, 5]. To our best knowledge, this is the first report of CSF p-tau elevation in patients with CNS VZV infection. This suggests that the biomarker profile of decreased $A\beta_{1-42}$, increased t-tau, and increased p-tau in CSF might be shared not only by CNS involvement of HSV infection but also CNS VZV involvement. This combination of biomarker changes, the so-called “AD signature”, has been considered to indicate the presence of AD pathology. In particular, high levels of p-tau at threonine181 have been reported to occur solely in AD and not in other neurodegenerative disorders or acute brain damage, such as acute brain infarction [6]. CSF p-tau elevation in the HSV/VZV group might be attributed to hyperphosphorylation of tau induced by APP mis-metabolism caused by herpetic infection [7], as similarly seen in the case of AD. Aside from the mechanism, we would like to emphasize the following: the fact that the biomarker profile in AD patients mimics that in patients with CNS HSV and VZV infections should be paid attention to as a confounding factor in the CSF biomarker-based diagnosis, where patients with recent reactivation of HSV or VZV might be misdiagnosed as preclinical AD.

Second, CSF sTREM2, a marker reflecting the extent of microglial inflammation, and GFAP, a marker of astrocyte damage, were elevated in the HSV and VZV groups compared with the control group. Those results are in line with previous observations [8]. Of note, these trends are also identical to those reported as biomarker changes in patients with AD [9] [10].

Third, only NfL levels were significantly correlated with the severity and a poor outcome after age adjustment in CSF biomarkers, while relationships between other biomarkers in CSF and those clinical symptoms disappeared after age adjustment if they were univariately significant. This suggests that the NfL concentration in CSF obtained for the diagnostic purpose on admission is the most powerful as a predictive marker of the severity and prognosis in patients with herpes virus infections among the molecules tested in this study. The correlation between CSF and blood NfL levels is reported to be tight in neurological disorders. In fact, serum levels of NfL were strongly associated to their matching CSF levels in our sample set (Supplementary Figure3) and consequently, ability of serum NfL to evaluate the severity and to predict prognosis were nearly equivalent to those of CSF NfL when we consider reduced statistical power due to some missing serum samples. Serum NfL might have potential as an efficient and useful blood-based biomarker for prediction of the severity and prognosis.

We acknowledge that the small sample size was a major limitation of this study. Furthermore, the short follow-up period may have weakened the statistical power to detect an association between the prognosis and biomarkers. In the future, it will be necessary to conduct large-scale case-control studies and prospective observations in order to validate the clinical significance of AD-related biomarkers in patients with CNS HSV and VZV infections.

In conclusion, this study demonstrated significantly decreased CSF $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}$/ $A\beta_{1-40}$ ratio and increased t-tau and p-tau in the HSV/VZV group. In addition, CSF sTREM2, a marker reflecting the extent of microglial inflammation, and GFAP, a marker of astrocytic damage, were elevated in the HSV/VZV group compared with the control group. The CSF profile of decreased $A\beta_{1-42}$ and increased t-tau and p-tau is known as the “AD signature”. In particular, elevated CSF p181-tau was recognized as

reflecting the presence of AD pathology. Moreover, increased sTREM2 and GFAP in CSF are also identical to those reported as biomarker changes in patients with AD. The fact that the biomarker profile in patients with CNS HSV and VZV infections mimics that in AD patients should be paid attention to as a pitfall in CSF biomarker-based diagnosis of AD. CSF NfL levels were significantly correlated with the disease severity and a poor outcome after age adjustment. The CSF NfL concentration on admission may be useful as a predictive marker of the severity and prognosis in patients with CNS HSV and VZV infections.

Abbreviations

CNS
central nervous system, AD:Alzheimer's disease, HSV:herpes simplex virus, VZV:varicella zoster virus, Aβ:amyloid β, t-tau:total tau, p-tau:tau phosphorylated at threonine 181, NfL:neurofilament light chain, p-NfH:phosphorylated neurofilament heavy chain, GFAP:glial fibrillary acidic protein, sTREM2:soluble triggering receptor expressed on myeloid cells 2, CSF:cerebrospinal fluid, MRI:magnetic resonance imaging, mRS:modified Rankin Scale, GCS:Glasgow coma scale, T2WI:T2-weighted imaging (T2WI), FLAIR:fluid-attenuated inversion recovery imaging.

Declarations

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Written informed consent from the participants was obtained when possible and, if not, from the nearest relative. The study protocols were approved by the University Ethics Committee (ERB-G-12, Kyoto Prefectural University of Medicine, Kyoto, Japan).

Consent for publication

Not applicable

Competing interests

The authors have no competing financial interests. Also, no non-financial conflicts of interest exist.

Authors contribution

F. K-M., T.O. and Y.F. assisted with patient enrollment, data analysis, and interpretation. H.T., F.K-M., and M.S. performed laboratory work and data analysis. D.A. and T.M. participated in review and revision of the manuscript. M.S., T.K. and T.T were involved with conceptualization and design of the study, patient enrollment, data collection, interpretation of the data, and review of the manuscript. All authors reviewed the drafts and approved the final version of the manuscript.

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Not applicable

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Figures

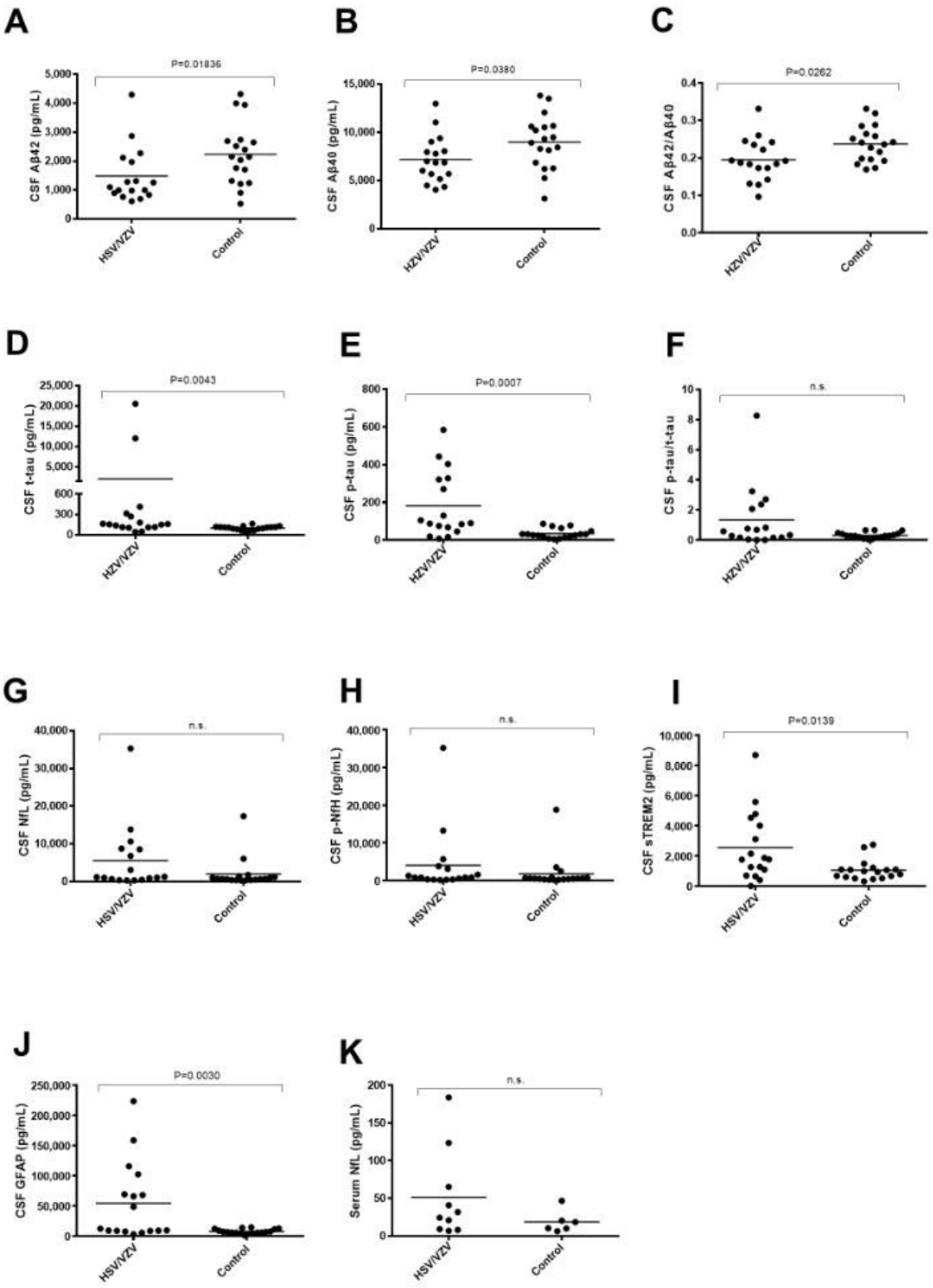

Figure 1

Comparison of biomarker concentrations between the HSV/VZV and control groups(A: CSF Aβ1-42, B: CSF Aβ1-40, C: CSF Aβ1-42/1-40 ratio, D: CSF t-tau, E:CSF p-tau, F: CSF p-tau/t-tau ratio, G: CSF NfL, H: CSF pNfH, I: CSF sTREM2, J: CSF GFAP, and K: serum NfL). The CSF (HSV/VZV group: n=17; control: n=18) and serum (HSV/VZV group: n=9; control: n=6) levels of those biomarkers in each individual are

shown as a black circle. Bars indicate median values. When a significant difference was observed between the groups, P-values were placed on n-shaped zig-zag lines. “n.s.” indicates “not significant”.

Supplementary Files

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- Supplementary2020902.docx