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Clinical utility of serum hepcidin and iron profile measurements in Alzheimer's disease

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ABSTRACT

Objectives: There are no generally accepted serum biomarkers for Alzheimer's disease (AD). We investigated the clinical usefulness of measuring the serum hepcidin levels and iron profile in patients with AD.
Materials & methods: The iron profile and hepcidin levels were measured in patients with AD ($N = 70$), minimal cognitive impairment (MCI, $N = 39$), and vascular dementia (VD, $N = 25$) and normal controls ($N = 124$). General cognitive tests were performed, and the relationships between cognition and hepcidin levels or the iron profile were assessed.
Results: Patients with AD had higher hepcidin values than those with MCI and VD and normal controls (median value: 39.00 vs. 30.81, 32.52, and 5.51 ng/ml, respectively, $P < 0.001$), and these differences were found in both men and women. The total iron-binding capacity was significantly lower in the AD group than in any other groups (308.0 vs. 332.0, 329.0, and 330.5 μg/dl, respectively, $P = 0.018$), and serum iron levels were lower in the AD group than controls (79.1 vs. 107.2 μg/dl, $P = 0.007$). Hepcidin levels were statistically significantly correlated with the clinical dementia rating (CDR, $P = 0.040$) with a Pearson's correlation coefficient of 0.253, and the patients with AD with a CDR value > 1 had significantly higher hepcidin values than those with a CDR value of 1 (65.26 vs. 23.49 ng/ml, $P = 0.020$).
Conclusion: The measurement of serum hepcidin levels and the iron profile in patients with early manifestations of cognitive functional loss might aid in the diagnosis of AD and the assessment of disease severity when combined with other diagnostic parameters.

1. Introduction

Alzheimer's disease (AD), a neurodegenerative disease, is characterized by slowly progressing memory loss and cognitive decline; it is the most common cause of dementia in the elderly population [1,2]. The diagnosis of AD is based on patient history, neuropsychological testing, and neuroimaging techniques, such as magnetic resonance imaging (MRI) or positron emission tomography (PET).

The classic pathological features of AD are plaque formation of amyloid β (Aβ) peptides and the accumulation of neurofibrillary tangles consisting of hyperphosphorylated tau protein [3]. The Aβ peptides are generated by cleavage of amyloid precursor protein (APP), an essential neuroprotective protein for neuronal growth, survival, and repair [4]. Several brain toxic materials such as manganese suppress the neuroprotective APP and lead to a profound accumulation of neurotoxic oxidative stress in brain tissue [5]. Under physiological conditions, APP is predominantly processed in a non-amyloidogenic manner, preclude Aβ production. In contrast, pathological APP processing generates neurotoxic Aβ peptide, the key event in the pathogenic cascade in AD [2]. Resulting amyloid plaques are known to be synaptotoxic, and Aβ oligomers (AβO), which are formed by two or more Aβ peptides, in the amyloid plaque are known to be more toxic than monomers [6]. mRNA encoding APP contains a sequence of iron-responsible element, iron metabolites are considered to modulate APP production [7].

In healthy aging, selective accumulation of iron occurs in several brain regions and cell types [8]. However, iron accumulation in brain greater than that in healthy aging occurs in many neurodegenerative diseases including AD and is often associated with oxidative stress [8]. With regard to the pathological mechanisms underlying AD, iron plays an important role in several mechanisms; in addition to APP modulation, redox-active iron is deposited near the Aβ plaque [9], Fe^{3+} binds to hyperphosphorylated tau protein, and is reduced to Fe^{2+}, which

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induces neurofibrillary tangle production [10].

Hepcidin, a liver-derived regulatory hormone, plays a major role in whole body iron homeostasis [11]. Hepcidin binds to an ion exporter, ferroportin, and induces its internalization and degradation, leading to decreased export of cellular iron [12]. Because ferroportin is located in macrophages, intestinal enterocytes, Kupffer cells, and hepatocytes, it regulates the transfer of iron to plasma mainly by these cells [13,14]. In addition to these cells, most cell types of brain also have ferroportin. And not only hepcidin, but also APP binds to ferroportin to facilitate neuronal iron export [15–17]. In these circumstances, hepcidin might directly or indirectly affect AD pathophysiology. Raha et al. revealed that hepcidin and ferroportin expression was reduced in the brain of patients compared to that of normal controls [13]. Thus, it is plausible that circulating hepcidin levels could also differ in patients with AD compared to normal controls.

Although several blood biomarkers for AD have been investigated and reported [18,19], there are still no generally accepted clinically useful blood biomarkers for AD; therefore, laboratory tests using blood samples from patients with AD are mainly conducted for the purpose of differential diagnosis. In addition, few studies have been conducted on serum hepcidin or AβO levels in patients with AD [11,18,20], and the clinical utility of these measurements is still poorly understood because of the small number of studies and the discrepant results among studies.

Here, we investigated the serum iron profile, and hepcidin and AβO levels, in patients with AD, mild cognitive impairment (MCI), vascular dementia (VD), and normal healthy subjects. The laboratory profiles were compared among the groups, and correlations of each variable to the general cognitive function scores were assessed to study the usefulness of these measurements for predicting disease severity.

2. Methods

2.1. Study subjects

The study subjects were outpatients with a clinical diagnosis of AD, MCI, or VD who visited the Neurology Department of Chung-Ang University Hospital, Seoul, Korea from September 2013 to June 2015. The number of subjects in the AD, MCI, or VD groups was 70 (including 44 women), 39 (including 27 women), and 25 (including 15 women), respectively. The patients were enrolled at the time when the diagnosis was made. In addition, 124 control subjects (including 72 women) also underwent neuropsychological tests, laboratory tests, and brain MRI scans, and they did not show any abnormalities on these neurological examinations. The median age of the AD, MCI, VD, and normal control groups was 76, 69, 79, and 74 years, respectively. Owing to possible interferences to the iron profile from hepcidin, the patients who had any evidence of iron deficiency anemia, acute illness, or C-reactive protein (CRP) levels ≥ 5 mg/l were excluded from the study.

2.2. Laboratory data

Residual blood samples were used; samples from the study subjects were collected after routine laboratory tests were performed and stored at -70 °C until analysis for this study. Hepcidin concentrations were measured with the Human Hepcidin Quantikine Enzyme-linked Immunosorbent Assay (ELISA) Kit (R&D Systems Inc., MN, USA). Ferritin, serum iron, unsaturated iron binding capacity (UIBC), and total iron binding capacity (TIBC) were measured using a chemistry analyzer (AU5800, Beckman Coulter, Inc., CA, USA). Serum $A\beta_{42}$ oligomers levels were measured using the Biosensis Oligomeric Amyloid-β ELISA kit (Biosensis Pty Ltd., Thebarton, South Australia). All analyses were performed according to the instructions from the manufacturers.

2.3. Clinical data

To assess the disease severity of patients with a cognitive disorder, medical records were reviewed for the time at which the patient's blood sample was collected. The results of the Korean version of the Mini-Mental State Examination (MMSE), Clinical Dementia Rating (CDR), Clinical Dementia Rating-Sum of Boxes (CDR-SOB), Global Deterioration Scale (GDS), Geriatric Depression Scale (GDpS), Barthel Index of Activities of Daily Living (BI-ADL), Seoul-Instrumental Activities of Daily Living (S-IADL), and Neuropsychiatric Inventory (NPI) were collected. Education level was also reviewed as the period of education.

2.4. Study design and statistical analyses

The Kolmogorov-Smirnov test was conducted to test the normality of the data. The laboratory or clinical data among the study groups were compared using a one-way analysis of variance (one-way ANOVA) or independent student's *t*-test for variables with a normal distribution, and the Kruskal-Wallis or Mann-Whitney *U* test for variables showing non-normal distributions. The Mann-Whitney U or Scheffe's multiple comparison tests were used for post-hoc analyses. A partial correlation analysis was used to examine the relationships between the laboratory data, adjusted for patient age and education level. Statistical analyses were performed using SPSS software, version 19 (SPSS, Chicago, IL, USA), and a P value of ≤ 0.05 was considered statistically significant.

2.5. Ethics statement

This case-control study was approved by the Chung-Ang University Hospital Institutional Review Board (IRB), and the need for informed consent was waived according to the IRB policy. The IRB approval number was C2013142(1102).

3. Results

The basic characteristics and general cognitive function test results for the study subjects are listed in Table 1. Sex ratio, median education levels, proportion of chronic disease including hypertension and diabetes mellitus, medical history of stroke and brain injury, hemoglobin levels, and CRP levels did not differ among the study groups. Patients with MCI showed milder cognitive function loss than any other group ($P < 0.05$), with the exception of the GDpS and BI-ADL results, and it was reasonable to define these patients as having “mild” cognitive impairment.

Comparisons of the iron profile, including hepcidin and AβO, among the AD, MCI, VD, and normal control groups are shown in Table 2 and Fig. 1. Serum hepcidin levels were statistically different among the groups. The patients with AD had significantly higher serum hepcidin values than the MCI, VD, and control groups (39.00 vs. 30.81, 32.52, and 5.51 ng/ml, respectively, $P > 0.001$). The serum hepcidin levels were higher in the MCI group than in the VD and normal control groups, although the values were lower than those in patients with AD. These differences were also found in the male and female subgroup analyses.

Serum iron levels differed between the AD and control groups. Patients with AD showed significantly lower iron levels compared to controls (79.1 vs. 107.2 μg/dl, $P = 0.007$). The TIBC level in patients with AD was significantly lower than in the MCI, VD, and control groups (308.0 vs. 332.0, 329.0, and 330.5 μg/dl, respectively, $P = 0.018$). This was also found in the female subgroup, but not in the male subgroup. UIBC, serum ferritin, and AβO levels did not show any differences among the groups.

3.1. Correlations among the clinical variables, iron profile and AβO levels

The correlations between the clinical variables and iron profile or AβO levels in patients with a cognitive disorder were investigated. Only serum iron levels in the patients with AD were significantly correlated

Table 1
Demographic characteristics and general cognitive function scores for the study subjects.

	AD (*N* = 70)	MCI (*N* = 39)	VD (*N* = 25)	Control (*N* = 124)	*P* value[a]	Kruskal-Wallis grouping[b]
Age (years)	76 [67, 83][c]	69 [61, 78]	79 [75, 83]	74 [68, 80]	< 0.001	A/B/A/A
Sex, M/F (N)	26/44	12/17	10/15	52/72	0.658	–
Education (years)	8.5 [4.5, 12.5]	8.8 [4.5, 12.8]	8.3 [4.3, 12.5]	NA	0.529	–
Hypertension	17.1%	17.9%	10.0%	16.7%	0.874	
DM	20.0%	23.1%	15.0%	13.3%	0.728	
Stroke history	7.1%	2.6%	5.0%	3.3%	0.722	
Brain injury history	2.9%	0.0%	0.0%	0.0%	0.462	
Hemoglobin (g/dl)	13.0 ± 0.9	12.9 ± 1.0	13.1 ± 1.1	13.2 ± 0.7	0.529	
CRP (mg/l)	1.30 [0.60, 2.00]	1.20 [0.95, 1.45]	1.25 [0.60, 1.9]	1.20 [0.90, 1.50]	0.339	
MMSE	17 [13, 19]	21.5 [20, 25]	15.5 [10.5, 19.5]	NA	< 0.001	A/B/A/–
CDR	1 [1, 2]	0.5 [0.5, 0.5]	1 [0.5, 2]	NA	< 0.001	A/B/A/–
CDR-SOB	5 [4.5, 7]	2.5 [2, 4]	5 [4, 10]	NA	< 0.001	A/B/A/–
GDS	4 [4, 4]	3 [3, 3.25]	4 [3, 5]	NA	0.000	A/B/A/–
GDpS	4 [3, 9]	6 [2, 10.5]	4 [2, 11]	NA	0.134	–
BI-ADL	20 [18, 20]	20 [20, 20]	18 [17, 20]	NA	0.096	–
S-IADL	15 [9.5, 26]	6 [2.25, 18.25]	27 [11.5, 39.5]	NA	0.001	A/B/A/–
NPI	17 [4, 34]	7 [2, 15]	18 [5, 34.5]	NA	0.015	A/B/A/–

Abbreviations: AD, Alzheimer's Disease; MCI, Mild Cognitive Impairment; VD, Vascular Dementia; CRP, C-Reactive Protein; MMSE, Korean version of Mini-Mental State Examination; CDR, Clinical Dementia Rating; CDR-SOB, Clinical Dementia Rating-Sum of Boxes; GDS, Global Deterioration Scale; GDpS, Geriatric Depression Scale; BI-ADL, Barthel Index of Activities of Daily Living; S-IADL, Seoul-Instrumental Activities of Daily Living; NA, Not Assessed; NPI, Neuropsychiatric Inventory.
[a] Statistically significant differences among groups were assessed using the Kruskal-Wallis test or Two-by-K crosstab analysis.
[b] The same letters indicate non-significant differences between groups based on the Mann-Whitney U test.
[c] Data are shown as "median [Q1, Q3]" or "mean ± standard deviation".

to the MMSE score ($r = 0.252$, $P = 0.040$ adjusted for age and education level). In the patients with AD, hepcidin ($r = 0.253$, $P = 0.040$) and TIBC ($r = 0.276$, $P = 0.025$) were significantly correlated with the CDR score. Partial correlations between other clinical variables and iron-related laboratory variables or AβO levels showed non-significant results.

Comparisons of iron-related laboratory variables or AβO levels according to the MMSE or CDR score were conducted and the results are listed in Table 3. There were no significant differences in the values among the groups with regard to MMSE scores > 20, 10–19, or 0–9. Patients with AD with a CDR value > 1 had significantly higher hepcidin levels than patients with a CDR value of 1 (65.26 vs. 23.49 ng/ml, $P = 0.020$, Fig. 2). These results suggest that patients with severe AD had higher hepcidin levels than patients with mild AD. Other iron profiles did not differ between the groups. For CDR-SOB, no significant differences were found between the groups.

In AD patients, serum hepcidin showed statistically significant correlations with ferritin ($r = 0.380$, $P = 0.001$), UIBC ($r = 0.281$, $P = 0.02$), and TIBC ($r = 0.321$, $P = 0.007$). Other iron profiles, AβO levels, and age did not show any significant correlation with serum hepcidin levels ($P > 0.05$).

Table 2
Comparison of the iron profile and amyloid-β oligomers among patients with Alzheimer's disease, mild cognitive impairment, vascular dementia, and the normal control group.

		AD (N = 70/26/44)[a]	MCI (N = 39/12/27)	VD (N = 25/10/15)	Control (N = 124/52/72)	*P* value[b]	Kruskal-Wallis or Scheffe grouping[c]
Hepcidin (ng/ml)	Total	39.00[d] [8.19, 107.48]	30.81 [8.67, 130.69]	32.52 [4.24, 124.18]	5.51 [2.49, 15.61]	< 0.001	A/B/B/C
	M	31.63 [6.95, 131.51]	20.17 [8.04, 124.62]	12.13 [1.67, 116.29]	5.21 [2.48, 12.32]	0.005	A/B/C/C
	F	42.37 [8.41, 88.00]	28.51 [8.67, 85.11]	33.77 [10.77, 135.16]	5.97 [2.51, 18.59]	< 0.001	A/B/B/C
Ferritin (ng/ml)	Total	116.2 [73.0, 199.1]	104.8 [72.4, 205.7]	96.1 [55.4, 181.8]	125.7 [84.4, 191.4]	0.097	–
	M	150.5 [69.3, 243.6]	164.7 [88.5, 254.2]	103.4 [54.0, 226.6]	134.1 [100.5, 240.8]	0.756	–
	F	113.0 [72.6, 155.9]	92.8 [56.0, 152.0]	90.1 [51.9, 121.4]	116.1 [79.5, 158.8]	0.447	–
Serum iron (μg/dl)	Total	79.1 ± 46.6[d]	98.3 ± 42.2	87.4 ± 48.4	107.2 ± 47.4	0.007	A/AB/AB/B
	M	87.2 ± 40.5	91.6 ± 56.5	85.4 ± 54.7	116.3 ± 50.1	0.199	–
	F	73.2 ± 42.8	73.6 ± 41.4	88.9 ± 45.2	100.7 ± 44.6	0.057	–
UIBC (μg/dl)	Total	222.5 [180.3, 261.0]	256.0 [200.0, 300.0]	239.0 [175.8, 289.0]	213.0 [176.5, 254.8]	0.127	–
	M	203.0 [156.8, 255.0]	201.5 [181.5, 270.0]	236.0 [166.0, 297.5]	188.5 [159.3, 227.5]	0.447	–
	F	232.0 [197.3, 267.3]	272.0 [220.0, 318.0]	246.0 [199.0, 280.0]	230.5 [196.0, 261.8]	0.071	–
TIBC (μg/dl)	Total	308.0 [276.8, 341.0]	332.0 [293.0, 381.0]	329.0 [293.0, 350.5]	330.5 [303.8, 367.0]	0.018	A/B/B/B
	M	301.0 [277.0, 326.5]	299.0 [245.8, 367.0]	324.0 [280.5, 348.0]	323.0 [291.3, 352.8]	0.220	–
	F	320.5 [276.3, 348.3]	337.0 [306.0, 399.0]	334.0 [293.0, 375.0]	337.0 [314.5, 375.0]	0.046	A/B/B/B
Aβ oligomer (ng/ml)	Total	0.03 [0.03, 0.24]	0.06 [0.03, 0.91]	0.03 [0.03, 0.43]	0.03 [0.03, 1.99]	0.148	–
	M	0.03 [0.03, 0.11]	0.04 [0.03, 0.33]	0.03 [0.03, 7.75]	0.03 [0.03, 1.96]	0.793	–
	F	0.03 [0.03, 1.44]	0.31 [0.03, 1.63]	0.03 [0.03, 0.36]	0.03 [0.03, 2.09]	0.561	–

Abbreviations: AD, Alzheimer's Disease; MCI, Mild Cognitive Impairment; VD, Vascular Dementia; UIBC, Unsaturated Iron Binding Capacity; TIBC, Total Iron Binding Capacity; Aβ oligomer, amyloid-β oligomer.
[a] Study numbers are shown as (N = total/male/female).
[b] Statistically significant differences among groups were tested using the Kruskal-Wallis test (for non-parametric distribution variables) or a one-way ANOVA (for variables with a normal distribution).
[c] The same letters indicate non-significant differences between groups based on the Mann-Whitney U or Scheffe's multiple comparison tests.
[d] Data are shown as "median [Q1, Q3]" for non-parametric distribution variables, and as "mean ± standard deviation" (for the variables with a normal distribution).

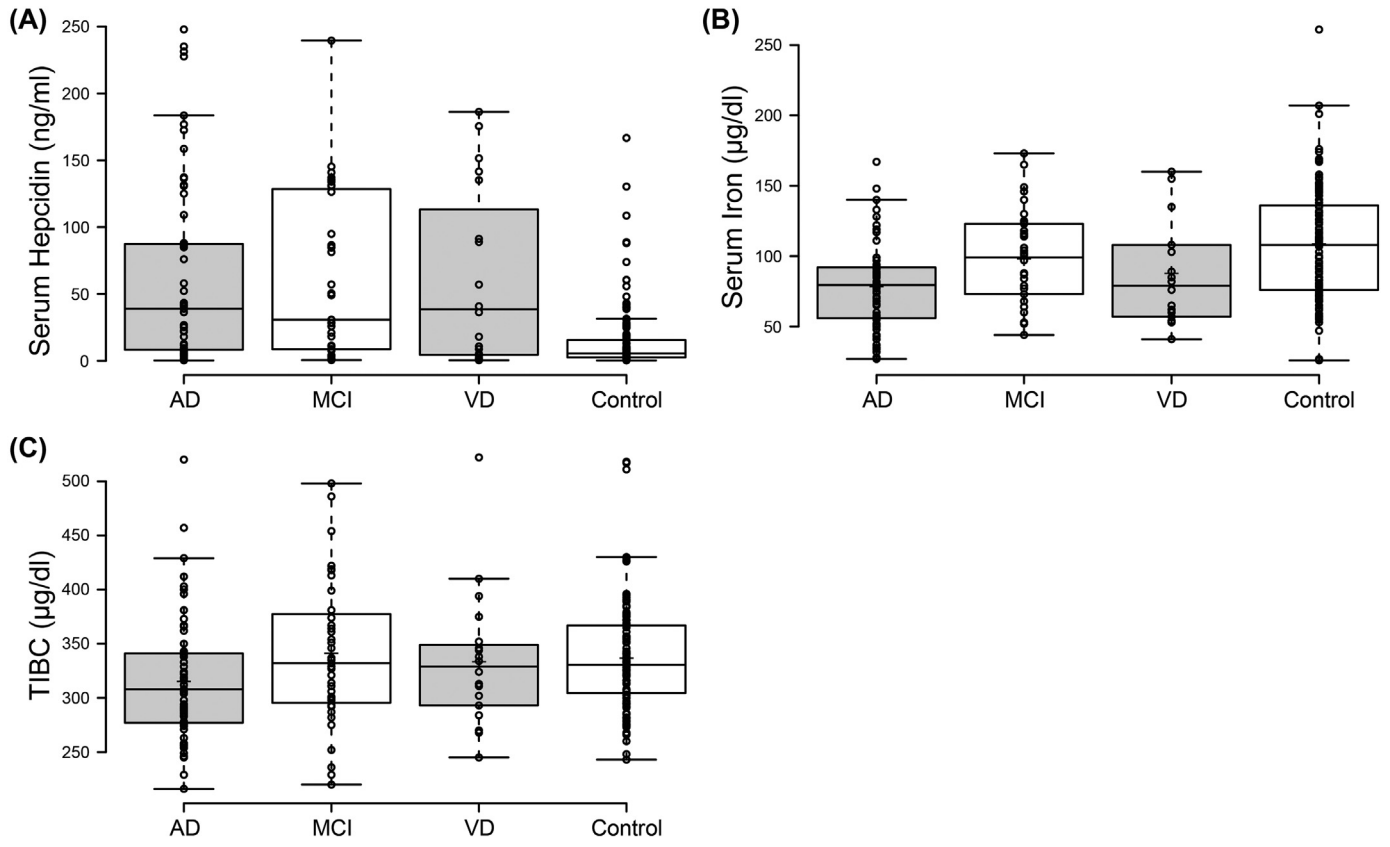

Fig. 1. Box-plots for serum hepcidin, iron, and TIBC among the study groups. Hepcidin and TIBC in AD patients were significantly different values than any other groups (A and C, $P < 0.001$ and $P = 0.007$, respectively), and iron was significantly lower than that of controls (B, $P = 0.018$).

4. Discussion

We investigated the clinical usefulness of serum iron profile measurements, including hepcidin, a less studied biomarker, for neurodegenerative disease [11,13,20] in patients with AD, MCI, and VD. Hepcidin levels were higher in patients with AD than in any other group; the values in the MCI and VD groups were also higher than in normal controls, but they were lower than in the patients with AD. Notably, these differences were found in both men and women. Circulating hepcidin levels can be attributed to iron store status, hypoxia, oxidative stress, and inflammation [21]. The inflammatory responses which are observed in AD brain can leads to increase in hepcidin level because hepcidin synthesis by hepatocytes is transcriptionally regulated by circulating interleukin-6 through the signal transducer and activator of transcription 3 (STAT-3) signaling pathway [13]. From this hypothesis, we analyzed CRP levels with study populations, but unexpectedly, the hepcidin levels were not significantly correlated to the CRP levels ($P = 0.75$, $r = 0.028$, from partial correlation analysis) and the CRP levels did not showed statistically significant different values among the study groups (Table 1). Neverthless, the inflammation in AD patients' brain cannot be completely ruled out from the causes of hepcidin elevation in AD because we excluded the patients who had CRP levels higher than 5.0 mg/l in the study and it could attribute to the insignificant results between CRP and hepcidin. Further investigations are need to clarify this scenario. In addition to inflammation, oxidative stress may also play a major role in the increase in serum hepcidin levels in patients with AD because the brains of patients with AD show significant oxidative damage that is associated with the abnormal accumulation of Aβ and the deposition of neurofibrillary tangles [22,23]. As hepcidin levels in the brain are decreased in patients with AD [13], hepcidin from the brain itself does not seem to represent the major source of the increased circulating hepcidin levels.

In the correlation analyses, hepcidin levels were significantly correlated with the CDR score in patients with AD. Additionally, in the subgroup analysis according to CDR levels within patients with AD, the patients with a CDR score > 1 had significantly higher hepcidin levels than the patients with a CDR score of 1. These findings suggest that serum hepcidin levels may represent a useful biomarker for predicting or assessing disease severity in patients with AD. However, the correlation coefficients were too low to argue that there is a strong correlation between hepcidin levels and CDR score or other clinical variables. Moreover, the MMSE and CDR-SOB did not show any significant correlation with serum hepcidin levels.

A discrepancy with previous studies exists. In the study performed by Sternberg et al., hepcidin levels were not significantly different between patients with AD and normal controls [11]; the hepcidin levels in patients with AD were higher than normal control subjects in the total group and in the male group, although there was no statistically significant difference, and the values were lower in patients with AD compared to controls in the female group. Moreover, they reported that hepcidin levels and CDR-SOB scores were significantly correlated, though this was not the case for the CDR scores. The reasons for this inconsistency may arise from the differences in the study population or measurement methods. All of our subjects were Asian, and although Sternberg et al. did not state the ethnic distribution of their study subjects, it is plausible that various ethnicities were involved because the patients were recruited in the United States of America. The differences in study number and sex distribution should also be considered. We included a much larger number of study patients and control subjects than previous studies, and we had more women in our study, which contrasted previous study groups. The ELISA kits used for serum hepcidin measurement differed also. However, these differences may not be the exact reason for the discrepancies between the results. Further evaluations with a larger number of patients are needed to fully

Table 3
Comparison of iron profiles and amyloid-β oligomer levels among patients with Alzheimer's disease subdivided by clinical variable scores.

	MMSE				CDR			CDR-SOB		
	> 20 (N = 12)	*10–19 (N = 53)*	*0–9 (N = 5)*	*P*[a]	*1 (N = 38)*	*> 1 (N = 22)*	*P*[b]	*0–9 (N = 59)*	*> 9 (N = 8)*	*P*[b]
Hepcidin (ng/ml)	42.09 [12.16, 124.97][c]	36.27 [8.10, 86.80]	42.02 [9.96, 154.52]	0.304	23.49 [7.13, 78.29]	65.25 [33.55, 132.16]	0.020	36.41 [7.91, 87.85]	42.37 [28.79, 87.31]	0.484
Ferritin (ng/ml)	127.2 [74.8, 191.3]	113.9 [73.7, 199.4]	127.9 [36.4, 269.6]	0.919	119.4 [70.9, 258.7]	122.9 [84.3, 158.3]	0.658	113.9 [72.1, 200.0]	135.4 [101.4, 171.0]	0.362
Serum iron (μg/dl)	94.5 ± 45.3[c]	91.5 ± 44.4	71.0 ± 30.6	0.542	90.2 ± 46.8	104.8 ± 37.5	0.178	92.3 ± 46.0	82.1 ± 36.4	0.476
UIBC (μg/dl)	206.0 [136.5, 254.5]	225.0 [182.5, 259.0]	261.0 [227.5, 309.0]	0.434	220.0 [174.3, 263.0]	218.0 [179.5, 252.2]	0.458	220.0 [175.0, 261.0]	221.5 [195.5, 257.0]	0.800
TIBC (μg/dl)	306.0 [259.3, 354.8]	308.0 [277.5, 339.5]	340.0 [284.0, 390.5]	0.712	307.0 [276.5, 338.8]	293.0 [260.7, 324.8]	0.250	308.0 [275.0, 341.0]	293.0 [279.2, 317.3]	0.256
Aβ oligomer (ng/ml)	0.03 [0.03, 0.25]	0.03 [0.03, 0.16]	1.04 [0.06, 2.18]	0.058	0.03 [0.03, 0.19]	0.03 [0.03, 0.13]	0.848	0.03 [0.03, 0.19]	0.11 [0.03, 0.46]	0.569

Abbreviations: MMSE, Korean version of Mini-Mental State Examination; CDR, Clinical Dementia Rating; CDR-SOB, Clinical Dementia Rating-Sum of Boxes; UIBC, Unsaturated Iron Binding Capacity; TIBC, Total Iron Binding Capacity; Aβ oligomer, amyloid-β oligomer.

[a] Statistically significant differences among groups were assessed using the Kruskal-Wallis Test (for non-parametric distribution variables) or a one-way ANOVA (for variables with a normal distribution).

[b] Statistically significant differences among groups were assessed using the Mann-Whitney U (for non-parametric distribution variables) or independent student's *t*-test (for variables with a normal distribution).

[c] Data are shown as "median [Q1, Q3]" for non-parametric distribution variables, and as "mean ± standard deviation" (for variables with a normal distribution).

Fig. 2. Serum hepcidin level between the AD patients with CDR of 1 and the AD patients with CDR values higher than 1. Patients group of CDR value > 1 had significantly higher hepcidin levels than patients with a CDR value of 1 ($P = 0.020$).

assess the correlation between serum hepcidin levels and disease severity.

We found decreased serum iron levels in patients with AD compared to normal controls. Additionally, although there were no statistically significant differences, the total groups of patients with MCI and VD had lower serum iron levels than normal controls. This was also reported by Hare et al. [24]. The authors found a significant decrease in transferrin-associated iron in AD that was missed by routine pathological tests of transferrin saturation, and concluded that transferrin desaturation could be the cause of the decreased serum iron levels observed in patients with AD. In addition, because hepcidin is an iron regulatory hormone [25], it is also possible that the iron levels could have been down-regulated by the increased serum hepcidin levels. TIBC values were lower in patients with AD than normal controls in the total group and in the female group. This might be caused by the upregulated hepcidin levels, because hepcidin was previously shown to down-regulate TIBC levels [26]. This may also be evidence of anemia of chronic disease (ACD) induced by AD itself or other co-morbidities. As TIBC is calculated as the sum of serum iron levels and UIBC, decreased iron would also affect the TIBC results. In summary, the upregulated hepcidin levels may result from pathophysiological mechanisms underlying AD, such as oxidative stress and inflammation, and might affect serum iron and TIBC levels in combination with various other factors. Measurements of hepcidin and the iron profile in patients with AD may aid in diagnosing patients with early manifestations of AD. Serum is easily obtainable and the results of the measurements are relatively easily interpreted. Within the iron profile, ferritin and UIBC did not show any significant difference between the patients with cognitive impairment and normal controls. Serum ferritin levels in patients with AD still represent a source of controversy. Brain ferritin can seep out from the brain to the blood through the leaky blood-brain barrier in patients with AD or blood ferritin may be elevated itself by the increased inflammatory response in AD [27,28]. Despite these assumptions, the results of studies that investigated serum ferritin in patients with AD are inconsistent. In the study by Sternberg et al., the serum ferritin level was higher in the total and male AD and MCI groups than in normal controls [11]. They concluded that the cause of the elevated serum ferritin levels in patients with AD might arise as a result of inflammation. In the current study, we did not find any evidence to support this scenario, and our results were concordant with the results of the study conducted by Fischer et al. who concluded that levels of ferritin were not significantly different in patients with AD compared to controls [29]. We assumed that the discrepancy between our results and Sternberg et al.'s also come from the sex composition of the study subjects similar to the results of hepcidin. Although it was statistically insignificant, ferritin increased in the serum of male AD patients

relative to normal subjects while it decreased in women. Thus, if there were more male AD subjects in our study, the results would be somewhat different.

Two Aβ peptides, $Aβ_{40}$ and $Aβ_{42}$ monomers, are the most studied biomarkers for AD. However, several studies examining the serum levels of these Aβ peptides and their ratio in patients with AD and normal controls have yielded conflicting results, even though few cross-sectional studies report higher Aβ peptide levels in patients with AD than in controls [11,30–33]. Here we assessed circulating $Aβ_{42}$ oligomers levels as the studies investigating serum AβO levels in AD are limited in the literature and have so far been inconclusive [18,34]. However, we found no meaningful differences in AβO values or correlations with general cognitive scores. Thus, the measurement of AβO levels in patients with AD is not clinically useful. In addition, we also measured AβO levels after incubating the blood sample for 72 h at 37 °C to compare them to the values before incubation. This was conducted based on the hypothesis that AβO levels would increase after incubation in samples from patients with AD compared to samples from patients with other diseases or normal controls, as other studies have revealed that after incubating non-oligomeric $Aβ_{42}$-spiked serum, AβO levels increased much more in samples from patients than from controls [18]. However, although we did not spike non-oligomeric amyloid peptide into our samples, no changes in serum AβO levels were found post-incubation. Serum AβO measurements do not represent a reliable or clinically useful biomarker for AD.

This study has several limitations. As almost all patients survived during the study period, autopsies to confirm a diagnosis of AD were not conducted. Furthermore, autopsy with the study subjects in the future was also not expected because of cultural reluctance for the autopsy procedures in Korea. Another limitation was that we did not measure other laboratory profiles, such as transferrin saturation, or serum $Aβ_{40}$ and $Aβ_{42}$ monomer levels. Finally, the numbers of patients included in the MCI and VD groups were relatively small.

In summary, serum hepcidin, iron, and TIBC levels in patients with AD were significantly different from those in controls, and they were significantly correlated with disease severity. Serum hepcidin levels and iron profile measurements, in combination with other diagnostic parameters, in patients with early manifestations of cognitive function loss may be helpful for the diagnosis of AD and for assessing disease severity.

Data sharing statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Author contribution

Research conception & design: Kim HR. Data acquisition: Lim YK, Kweon OJ, Youn YC. Data analysis and interpretation: Kweon OJ, Lim YK, Youn YC. Statistical analysis: Lim YK, Kweon OJ. Drafting of the manuscript: Kweon OJ. Critical revision of the manuscript: Kim HR, Lee MK, Youn YC. Receiving grant: Kim HR. Approval of final manuscript: all authors.

Declaration of Competing Interest

None.

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References

[1] M.O. Grimm, J. Mett, T. Hartmann, The impact of vitamin E and other fat-soluble vitamins on Alzheimer s disease, Int. J. Mol. Sci. 17 (11) (2016).
[2] D.J. Selkoe, Alzheimer's disease: genes, proteins, and therapy, Physiol. Rev. 81 (2) (2001) 741–766.
[3] S. Oshiro, M.S. Morioka, M. Kikuchi, Dysregulation of iron metabolism in Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, Adv. Pharmacol. Sci. 2011 (2011) 8.
[4] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science (New York, N.Y.) 297 (5580) (2002) 353–356.
[5] V. Venkataramani, T.R. Doeppner, D. Willkommen, C.M. Cahill, Y. Xin, G. Ye, et al., Manganese causes neurotoxic iron accumulation via translational repression of amyloid precursor protein and H-ferritin, J. Neurochem. 147 (6) (2018) 831–848.
[6] G.M. Shankar, S. Li, T.H. Mehta, A. Garcia-Munoz, N.E. Shepardson, I. Smith, et al., Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory, Nat. Med. 14 (8) (2008) 837–842.
[7] L. Zecca, M.B. Youdim, P. Riederer, J.R. Connor, R.R. Crichton, Iron, brain ageing and neurodegenerative disorders, Nat. Rev. Neurosci. 5 (11) (2004) 863–873.
[8] R.J. Ward, F.A. Zucca, J.H. Duyn, R.R. Crichton, L. Zecca, The role of iron in brain ageing and neurodegenerative disorders, Lancet Neurol. 13 (10) (2014) 1045–1060.
[9] M.A. Smith, P.L. Harris, L.M. Sayre, G. Perry, Iron accumulation in Alzheimer disease is a source of redox-generated free radicals, Proc. Natl. Acad. Sci. U. S. A. 94 (18) (1997) 9866–9868.
[10] A. Yamamoto, R.W. Shin, K. Hasegawa, H. Naiki, H. Sato, F. Yoshimasu, et al., Iron (III) induces aggregation of hyperphosphorylated tau and its reduction to iron (II) reverses the aggregation: implications in the formation of neurofibrillary tangles of Alzheimer's disease, J. Neurochem. 82 (5) (2002) 1137–1147.
[11] Z. Sternberg, Z. Hu, D. Sternberg, S. Waseh, J.F. Quinn, K. Wild, et al., Serum hepcidin levels, iron dyshomeostasis and cognitive loss in Alzheimer's disease, Aging Dis. 8 (2) (2017) 215–227.
[12] E. Nemeth, M.S. Tuttle, J. Powelson, M.B. Vaughn, A. Donovan, D.M. Ward, et al., Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization, Science (New York, N.Y.) 306 (5704) (2004) 2090–2093.
[13] A.A. Raha, R.A. Vaishnav, R.P. Friedland, A. Bomford, R. Raha-Chowdhury, The systemic iron-regulatory proteins hepcidin and ferroportin are reduced in the brain in Alzheimer's disease, Acta Neuropathol. Commun. 1 (2013) 55.
[14] A. Donovan, C.A. Lima, J.L. Pinkus, G.S. Pinkus, L.I. Zon, S. Robine, et al., The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis, Cell Metab. 1 (3) (2005) 191–200.
[15] J.A. Duce, A. Tsatsanis, M.A. Cater, S.A. James, E. Robb, K. Wikhe, et al., Iron-export ferroxidase activity of beta-amyloid precursor protein is inhibited by zinc in Alzheimer's disease, Cell. 142 (6) (2010) 857–867.
[16] R.C. McCarthy, D.J. Kosman, Mechanisms and regulation of iron trafficking across the capillary endothelial cells of the blood-brain barrier, Front. Mol. Neurosci. 8 (2015) 31.
[17] A. Tsatsanis, S. Dickens, J.C.F. Kwok, B.X. Wong, J.A. Duce, Post translational modulation of beta-amyloid precursor protein trafficking to the cell surface alters neuronal iron homeostasis, Neurochem. Res. 44 (6) (2019) 1367–1374.
[18] S.S.A. An, B.S. Lee, J.S. Yu, K. Lim, G.J. Kim, R. Lee, et al., Dynamic changes of oligomeric amyloid beta levels in plasma induced by spiked synthetic Abeta42, Alzheimers Res. Ther. 9 (1) (2017) 86.
[19] C. Wang, Y. Cui, J. Yang, J. Zhang, D. Yuan, Y. Wei, et al., Combining serum and urine biomarkers in the early diagnosis of mild cognitive impairment that evolves into Alzheimer's disease in patients with the apolipoprotein E 4 genotype, Biomarkers 20 (1) (2015) 84–88.
[20] M. Victor, H. S, P. Julia, V. Vasil, P. M, T. Latchezar, et al., The role of iron homeostasis in Alzheimer's disease, J. Alzheimers Neurodegener. 3 (011) (2017).
[21] A.E. Armitage, L.A. Eddowes, U. Gileadi, S. Cole, N. Spottiswoode, T.A. Selvakumar, et al., Hepcidin regulation by innate immune and infectious stimuli, Blood. 118 (15) (2011) 4129–4139.
[22] Y. Christen, Oxidative stress and Alzheimer disease, Am. J. Clin. Nutr. 71 (2) (2000) (621s–9s).
[23] W.J. Huang, X. Zhang, W.W. Chen, Role of oxidative stress in Alzheimer's disease, Biomed. Rep. 4 (5) (2016) 519–522.
[24] D.J. Hare, J.D. Doecke, N.G. Faux, A. Rembach, I. Volitakis, C.J. Fowler, et al., Decreased plasma iron in Alzheimer's disease is due to transferrin desaturation, ACS Chem. Neurosci. 6 (3) (2015) 398–402.
[25] T. Ganz, Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation, Blood. 102 (3) (2003) 783–788.
[26] H. Drakesmith, A.M. Prentice, Hepcidin and the iron-infection axis, Science (New York, N.Y.) 338 (6108) (2012) 768–772.
[27] Y. Pan, J.A. Nicolazzo, Impact of aging, Alzheimer's disease and Parkinson's disease on the blood-brain barrier transport of therapeutics, Adv. Drug Deliv. Rev. 135 (2018) 62–74.
[28] S. Janelidze, J. Hertze, K. Nagga, K. Nilsson, C. Nilsson, M. Wennstrom, et al., Increased blood-brain barrier permeability is associated with dementia and diabetes but not amyloid pathology or APOE genotype, Neurobiol. Aging 51 (2017) 104–112.

[29] P. Fischer, M.E. Gotz, W. Danielczyk, W. Gsell, P. Riederer, Blood transferrin and ferritin in Alzheimer's disease, Life Sci. 60 (25) (1997) 2273–2278.
[30] N. Pomara, L.M. Willoughby, J.J. Sidtis, P.D. Mehta, Selective reductions in plasma Abeta 1-42 in healthy elderly subjects during longitudinal follow-up: a preliminary report, Am. J. Geriatr. Psychiatr. 13 (10) (2005) 914–917.
[31] M.C. Irizarry, Biomarkers of Alzheimer disease in plasma, NeuroRx 1 (2) (2004) 226–234.
[32] N.R. Graff-Radford, J.E. Crook, J. Lucas, B.F. Boeve, D.S. Knopman, R.J. Ivnik, et al., Association of low plasma Abeta42/Abeta40 ratios with increased imminent risk for mild cognitive impairment and Alzheimer disease, Arch. Neurol. 64 (3) (2007) 354–362.
[33] R. Mayeux, L.S. Honig, M.X. Tang, J. Manly, Y. Stern, N. Schupf, et al., Plasma A[beta]40 and A[beta]42 and Alzheimer's disease: relation to age, mortality, and risk, Neurology. 61 (9) (2003) 1185–1190.
[34] W. Xia, T. Yang, G. Shankar, I.M. Smith, Y. Shen, D.M. Walsh, et al., A specific enzyme-linked immunosorbent assay for measuring beta-amyloid protein oligomers in human plasma and brain tissue of patients with Alzheimer disease, Arch. Neurol. 66 (2) (2009) 190–199.