J. Aichi Med. Univ. Assoc.
Vol. 50, No. 1, pp. 1–11 (March 15, 2023)

The Utility of Serum Copper/Zinc Ratio for Evaluating the Pathophysiology of Alzheimer's Disease

Miku Nakagawa[1], Soutarou Taguchi[1)2)], Masayuki Izumi[3], Manabu Doyu[1]

[1] *Department of Neurology, Aichi Medical University Hospital, Aichi, Japan*
[2] *Parkinson's Disease Advanced Therapy Center, Aichi Medical University Hospital, Aichi, Japan*
[3] *College of Nursing, Aichi Medical University, Aichi, Japan*

The serum copper/zinc ratio has recently been identified as a biomarker of oxidative stress, potentially attributable to Alzheimer's disease (AD). We aimed to determine if this ratio would be useful for evaluating the pathophysiology of AD. We recruited 45 patients with AD and 45 control patients with chronic ischemic stroke. The latter participants had no difficulties with activities of daily living and no cognitive impairment. Neither group had any medical history known to affect the serum concentrations of trace elements and there was no difference in gender composition and nutritional status between the groups. The serum copper/zinc ratio was significantly higher in the AD group (median 1.57 [inter-quartile range 1.40, 1.88]) than the control group (median 1.40 [inter-quartile range 1.18, 1.75]) ($p=0.0187$). This ratio correlated with the degree of medial temporal lobe atrophy, as a typical AD lesion ($r=0.3538$, $p=0.0293$), and with cognitive deficit ($r=-0.651$, $p=0.0160$) especially in the patients with severe atrophy. These results suggest that the serum copper/zinc ratio could be a candidate indicator for evaluating the pathophysiology of AD.

Key words: Alzheimer's disease, copper, zinc, serum, trace element

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease which manifests with short-term memory loss in the early stage and leads to executive dysfunction and visual spatial abnormalities in the later stage. The patients' abilities to perform activities of daily living decline, and the patients may require nursing home placement. In addition, social problems, such as patients wandering and going missing, lead to the increased risk of road traffic accidents. The total number of dementia patients in Japan is expected to reach around 7 million by 2025 including AD patients accounting for 70% of their cases[1]. The severe socio-economic impacts of the disease highlight the need for the early diagnosis, assessment, and therapeutic interventions.

Trace elements, such as copper (Cu) and zinc (Zn), are present in the human body in the smaller amounts than iron. They are absorbed from the gastrointestinal epithelial cells, circulate in the blood with carrier proteins, and accumulate intracellularly with transporters. These elements play several roles in the body: they are essential components of enzymes and also act as electron donors or acceptors in reduction or oxidation reactions[2)3)].

Although many studies have examined the relationships between AD and serum concen-

Received February 15, 2023; Accepted March 22, 2023

trations of Cu and Zn[4)–8)], the results remain controversial. Our analysis of previous data suggested that the serum Cu/Zn ratio was elevated in AD patients[5)–8)]. This ratio has recently been focused on as a biomarker of oxidative stress[9)]; however, its usefulness is unclear in the AD pathophysiology which involves oxidative stress. In the present study, we investigated the utility of the serum Cu/Zn ratio for evaluating the pathophysiology of AD.

MATERIALS AND METHODS

Subjects

We enrolled consecutively patients with AD who were diagnosed according to the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association criteria[10)] from March 2, 2020, to January 7, 2022 (AD group). We also enrolled patients with chronic ischemic stroke who had no difficulties with activities of daily living and no cognitive impairment (control group) (Table 1). Neither group had any medical history of hepatic, kidney, metabolic, or digestive diseases known to affect serum concentrations of trace elements, and there were no significant difference in age, gender composition, nutritional status (body weight, body mass index, serum total protein concentration, and serum albumin concentration), liver function (serum aspartate aminotransferase and alanine aminotransferase), renal marker (serum creatinine concentration), and inflammatory marker (C-reactive protein) between the two groups. Cognitive function was assessed using the Revised Hasegawa's Dementia Scale (HDS-R) and the Mini-Mental State Examination (MMSE). Both scores exceeded the cut-off values in the control group (HDS-R: cut-off 20 points, median 27 points [inter-quartile range (IQR) 25.8, 29]; MMSE: cut-off 24 points, median 27 points [IQR 25, 28])[11)12)],

Table 1. Characteristics of Alzheimer's disease and control subjects

Measure	AD group	Control group	p-value
Total (male : female)	45 (20 : 25)	45 (21 : 24)	0.8324
Age (yr.)	80 [IQR 76, 84]	77 [IQR 73, 81]	0.0644
Body weight (kg)	53 [IQR 47.2, 58]	58 [IQR 49, 65.5]	0.0627
BMI (kg/m^2)	21.5 [IQR 20.1, 24.0]	22.4 [IQR 20.1, 24.5]	0.3089
TP (g/dL)	7.1 [IQR 6.8, 7.43]	7 [IQR 6.55, 7.3]	0.1932
Alb (g/dL)	4.1 [IQR 3.85, 4.2]	4 [IQR 3.8, 4]	0.1955
AST (IU/L)	22 [IQR 17, 25]	21.5 [IQR 19, 29.3]	0.2223
ALT (IU/L)	16 [IQR 12, 20]	16.5 [IQR 14, 28]	0.1051
Cr (mg/dL)	0.725 [IQR 0.573, 0.858]	0.705 [IQR 0.61, 0.843]	0.7557
CRP (mg/dL)	0.06 [IQR 0.04, 0.108]	0.09 [IQR 0.05, 0.14]	0.1351
MMSE (points)	20.5 [IQR 17, 23.3]	27 [IQR 25, 28]	$p<0.001$
HDS-R (points)	19 [IQR 15, 24]	27 [IQR 25.8, 29]	$p<0.001$

AD: Alzheimer's disease, Alb: albumin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BMI: body mass index, Cr: creatinine, CRP: C-reactive protein, HDS-R: revised Hasegawa's Dementia Scale, IQR: inter-quartile range, MMSE: Mini Mental State Examination and TP: total protein.

The AD and control groups included 45 participants each. There were not significantly differences in age, gender composition, nutritional status, serum liver enzyme activities, renal marker, and inflammatory marker between the groups. The HDS-R and MMSE scores exceeded the cut-off values in the control group but were significantly lower in the AD group.

while the scores in the AD group (HDS-R: 18 points [IQR 15, 19]; MMSE: 20.5 points [IQR 17, 23.3]) were significantly lower ($p<0.001$, respectively) (Table 1).

Measurement of serum trace element concentrations

Blood samples were collected from fasting subjects and centrifuged at 2,500×g for 8 minutes to obtain serum. The serum Cu concentration was determined by direct colorimetric assay using a Quick Auto Neo Cu kit (Shino-Test Corp., Tokyo, Japan)[13] and serum Zn was determined by atomic absorption spectrophotometry using an Espa-Zn II kit (Nipro Corp. Osaka, Japan)[14].

Neuroradiological analysis

Each subject was scanned by plain magnetic resonance imaging using 1.5 Tesra Vision Plus imager (SIMENS Corp. Tokyo, Japan). The degree of atrophy in the medial temporal lobe was assessed quantitatively as the mean Z-score in the region with Voxel-Based Specific Regional Analysis System for Alzheimer's Disease software (VSRAD® advance) (Eisai Corp. Tokyo, Japan)[15].

Subgroup analysis in AD patients

Atrophy of the medial temporal lobe, including the hippocampus, amygdala and entorhinal cortex, is a typical feature in patients with AD[16]. We carried out subgroup analysis to investigate how medial temporal atrophy affected the serum concentrations of Cu and Zn. We divided AD patients into subgroups according to the severity of atrophy: severe atrophy subgroup (Z-score$>$2) vs. non-severe atrophy subgroup (Z-score$\leqq$2). There were no significant differences between the subgroups in terms of age, gender composition, nutritional status, liver function, renal markers, and inflammatory marker (Table 2). The MMSE scores (median 17 points [IQR 15.5, 20]) and HDS-R scores (median 16 points [IQR 15, 18]) in the severe atrophy subgroup were significantly lower than those in the non-severe atrophy subgroup (median 23

Table 2. Characteristics of the Alzheimer's disease patients according to severity of medial temporal lobe atrophy

Measure	Severe atrophy subgroup	Non-severe atrophy subgroup	p-value
Total (male : female)	20 (9 : 11)	25 (11 : 14)	0.7423
Age (yr.)	82 [IQR 78.5, 84.5]	77 [IQR 70.75, 84]	0.1429
Body weight (kg)	51 [IQR 46.5, 59]	56.5 [IQR 43.5, 43.5]	0.8453
BMI (kg/m^2)	21.43 [19.55, 23.66]	22.30 [IQR 21.16, 24.78]	0.1978
TP (g/dL)	7 [IQR 6.7, 7.6])	7.2 [6.85, 7.5]	0.4749
Alb (g/dL)	4 [IQR 3.8, 4.2]	4.1 [IQR 3.975, 4.4]	0.2459
AST (IU/L)	20 [IQR 17, 24]	23 [IQR 17.5, 28]	0.5784
ALT (IU/L)	14.5 [IQR 11.25, 20]	18 [IQR 13, 20]	0.5118
Cr (mg/dL)	0.765 [IQR 0.5875, 0.9125]	0.64 [IQR 0.4925, 0.745]	0.1712
CRP (mg/dL)	0.06 [IQR 0.04, 0.1]	0.045 [IQR 0.03, 0.0725]	0.1475
MMSE (points)	17 [IQR 15.5, 20]	23 [IQR 21, 24]	0.0052
HDS-R (points)	16 [IQR 15, 18]	19 [IQR 17.5, 19]	0.0083

AD: Alzheimer's disease, Alb: albumin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BMI: body mass index, Cr: creatinine, CRP: C-reactive protein, HDS-R: revised Hasegawa's Dementia Scale, IQR: inter-quartile range, MMSE: Mini Mental State Examination and TP: total protein.

There were no significant differences between these subgroups in terms of age, gender composition, nutritional status, serum liver enzyme activities, renal marker, or inflammatory marker. The MMSE and HDS-R scores were significantly lower in the sever atrophy subgroup compared with the non-severe atrophy subgroup.

points [IQR 21, 24] and median 19 points [IQR 17.5, 19]) ($p<0.001$, respectively).

Statistical analysis

The data were analyzed using JMP version 14 (SAS Institute, Inc, NC, USA). The Shapiro-Wilk test was used to determine if variables showed a normal distribution. Continuous datasets were presented as mean and standard deviation and compared using ANOVA. Variables with a non-normal distribution were expressed as median and IQR and compared using Wilcoxon's rank-sum test. Categorical variables were presented as percentages and compared using χ^2 tests. Correlations between continuous variables were analyzed using Spearman's rank correlation coefficient. Multiple regression analysis was used to determine if the Z-score and HDS-R were exposure factors affecting the serum Cu/Zn ratio. The level of significance was set as $p<0.05$.

Ethics

This study was approved by the ethics board of Aichi Medical University (No. 2020-199). All participants provided written informed consent.

Declaration of conflicting interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and publication of this article.

RESULTS

The serum Cu/Zn ratio was significantly higher in the AD group (median 1.57 [IQR 1.40, 1.88]) than in the control group (median 1.40 [IQR 1.18, 1.75]) ($p=0.0187$). The serum Cu concentration tended to be higher in the AD group (median 113 μg/dL [IQR 103, 128]) than in the control group (median 105 μg/dL [IQR 91.5, 122]); however, this difference was not significant ($p=0.0656$). The serum Zn concentration was similar in the AD group (median 72 μg/dL [IQR 63, 79.5]) and the control group (median 74 μg/dL [IQR 69.5, 83.5]) ($p=0.0828$) (Fig. 1).

In the AD group, mean Z-score in the medial temporal lobe had positively significant correlation with the serum Cu/Zn ratio ($r=0.3538$, $p=0.0293$), but not with either serum Cu ($r=0.1510$, $p=0.4431$) or serum Zn ($r=-0.3124$, $p=0.1056$) alone (Fig. 2). The mean Z-score in the medial temporal lobe had negatively signi-

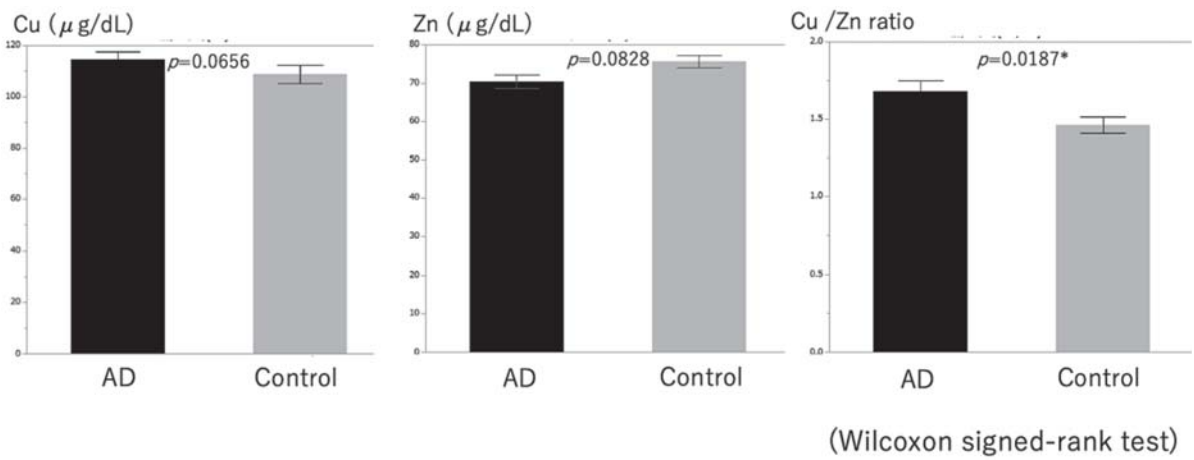

Fig. 1.
AD: Alzheimer's disease, Cu: serum copper concentration, Zn: serum zinc concentration, Cu/Zn ratio: the serum copper/zinc ratio
The serum Cu/Zn ratio was significantly higher in the AD group than in the control group, but there were no significant differences in serum Cu and Zn concentrations.

ficant correlations with each of the HDS-R ($r=-0.423$, $p=0.0053$) and MMSE scores ($r=-0.376$, $p=0.0142$) (Fig. 3). Nevertheless, the serum Cu and Zn concentrations and the Cu/Zn ratio were not correlated with the HDS-R ($r=-0.0632$, $p=0.852$; $r=0.2661$, $p=0.1344$; and $r=-0.1490$, $p=0.4414$; respectively) or MMSE scores ($r=-0.1764$, $p=0.358$; $r=0.2536$, $p=0.1462$; and $r=-0.0465$, $p=0.907$; respectively) in the AD group (Fig. 4).

Then we carried out subgroup analysis additionally to investigate how medial temporal atrophy affected the serum concentrations of Cu and Zn. The serum Cu/Zn ratio was significantly higher in the severe atrophy subgroup (median 1.667 [IQR 1.428, 1.976]) than in the non-severe atrophy subgroup (median 1.399 [IQR 1.217, 1.609]) ($p=0.0337$) (Fig. 5). The serum Cu concentration in the AD group showed a trend towards being higher in the severe atrophy subgroup (median 117 μg/dL [IQR 106.5, 127.5]) than in the non-severe atro-

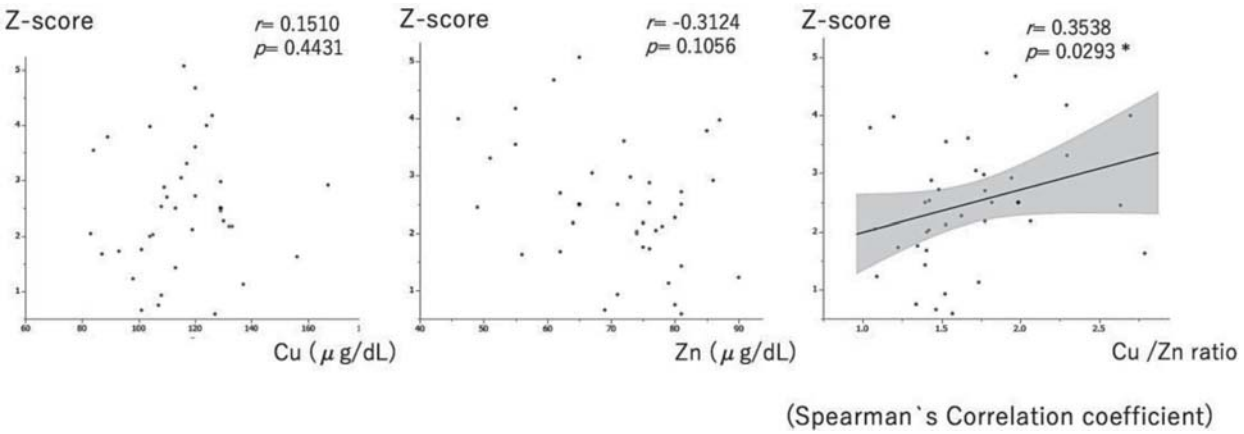

Fig. 2.
Cu: serum copper concentration, Zn: serum zinc concentration, Cu/Zn ratio: the serum copper/zinc ratio, Z-score: medial temporal lobe Z-score
In the AD group, the medial temporal lobe Z-score showed no correlation with serum Cu and Zn concentrations but was positively correlated with the serum Cu/Zn ratio.

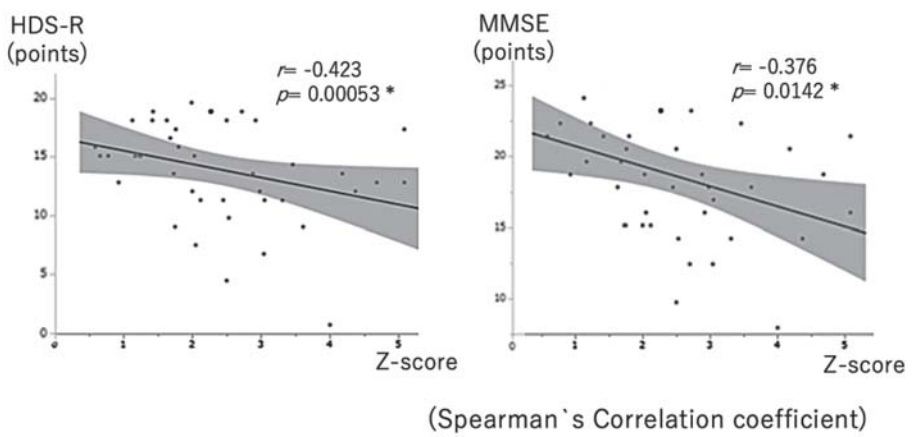

Fig. 3.
HDS-R: Revised Hasegawa's Dementia Scale, MMSE: Mini-Mental State Examination, Z-score: medial temporal lobe Z-score
The mean Z-score in the medial temporal lobe was negatively correlated with and the HDS-R and MMSE scores.

phy subgroup (median 104 μg/dL [IQR 96.75, 116.5]), but the difference was not significant ($p=0.1128$). There was no significant difference in serum Zn concentrations between the two subgroups (the severe atrophy subgroup: median 72 μg/dL [IQR 77.5, 61]; the non-severe atrophy subgroup: median 75.5 μg/dL [IQR 81, 69]; $p=0.1352$). In addition, there was a negative correlation between the serum Cu/Zn ratio and the HDS-R score in the severe atrophy sub-

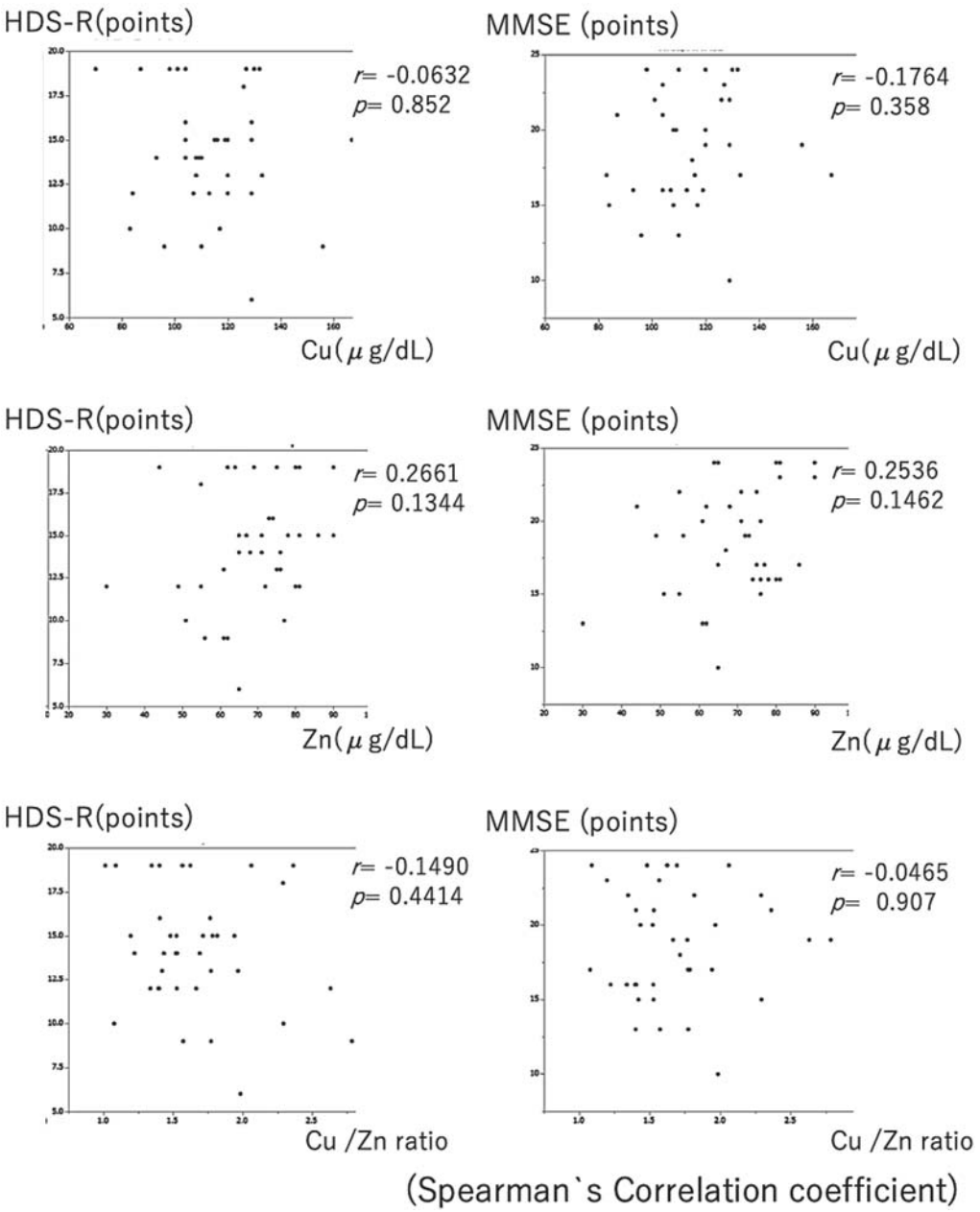

Fig. 4.

HDS-R: Revised Hasegawa's Dementia Scale, MMSE: Mini-Mental State Examination, Cu: serum copper concentration, Zn: serum zinc concentration, Cu/Zn ratio: the serum copper/zinc ratio

Serum Cu concentration, serum Zn concentration and the serum Cu/Zn ratio were not correlated with the HDS-R and MMSE scores in the AD group.

group ($r=-0.651$, $p=0.0160$), but no such relationship in the non-severe atrophy subgroup ($r=0.4294$, $p=0.1672$). There was no correlation between the MMSE score and the Cu/Zn ratio in either subgroup ($r=0.1689$, $p=0.336$; $r=0.0701$, $p=0.8277$; respectively) (Fig. 6). Multiple regression analysis showed that the serum Cu/Zn ratio was significantly affected by both the Z-score and the HDS-R score in the severe atrophy subgroup, with no multicollinearity of these two explanatory variables. This indicates that the degree of medial temporal lobe atrophy and the severity of AD independently affect the serum Cu/Zn ratio (Table 3).

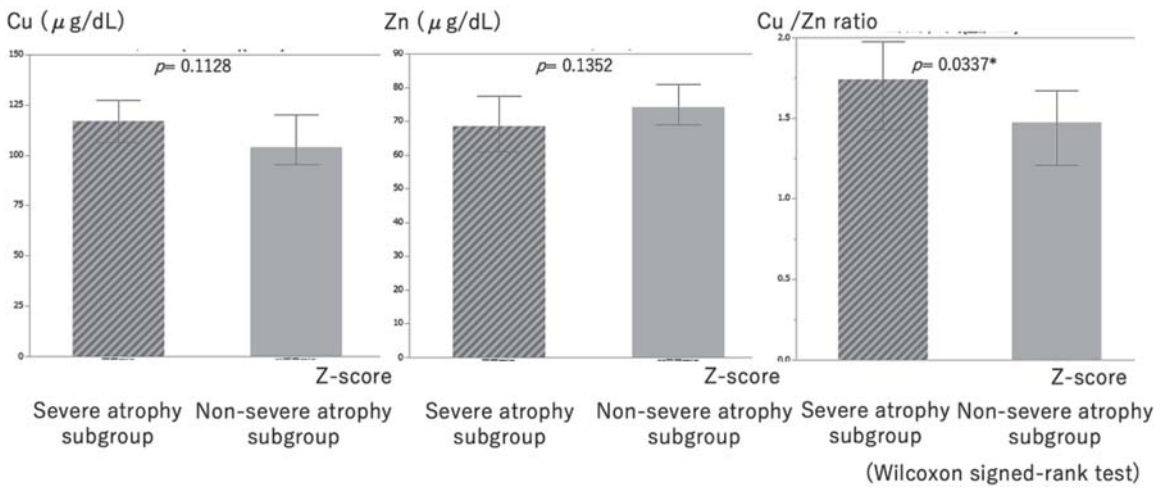

Fig. 5.
Cu: serum copper concentration, Zn: serum zinc concentration, Cu/Zn ratio: the serum copper/zinc ratio, Z-score: medial temporal lobe Z-score
There were no significant differences in serum Cu and Zn concentrations between these subgroups, but serum Cu/Zn ratio was higher in the Severe atrophy subgroup compared with the non-severe atrophy subgroup.

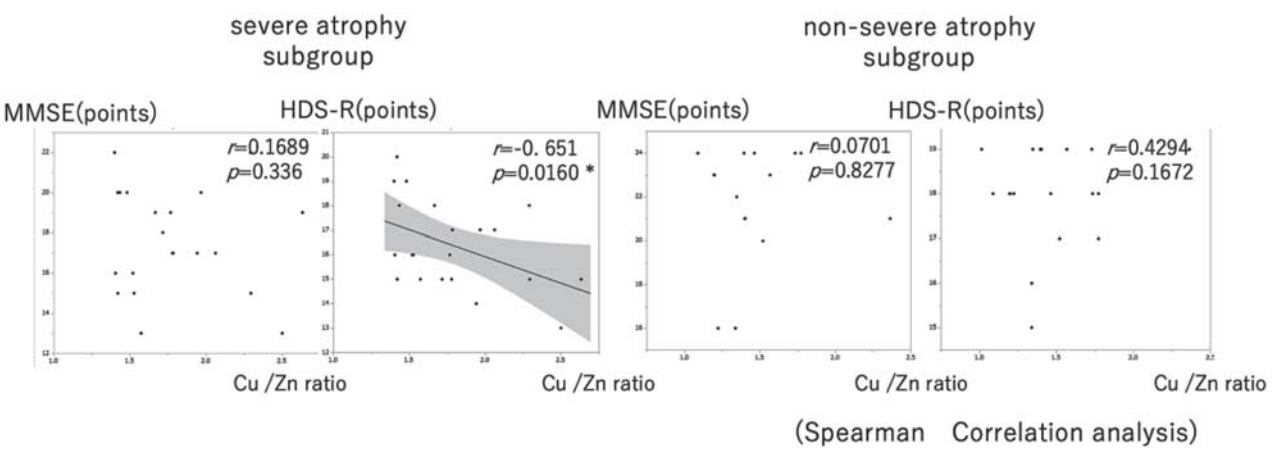

Fig. 6.
HDS-R: Revised Hasegawa's Dementia Scale, MMSE: Mini-Mental State Examination, Z-score: medial temporal lobe Z-score
There was a negative correlation between the serum Cu/Zn ratio and the score of the HDS-R score in the severe atrophy subgroup but not in the non-severe atrophy subgroup. There was no correlation between the MMSE score and the serum Cu/Zn ratio in either subgroup.

Table 3. Multiple regression analysis potential exposure factors affecting serum Cu/Zn ratio

Explanatory variable	B	SE	p-value	CI		VIF
				upper bound	lower bound	
Z-score	0.65	0.06862	0.0005	0.1704	0.4669	1.005
HDS-R	−0.5244	0.01154	0.0024	−0.0682	−0.0183	1.005

B: standardized beta coefficient, CI: confidence intervals, HDS-R: revised Hasegawa's Dementia Scale, SE: standard error and VIF: variance inflation factor.
Multiple regression analysis showed that the serum Cu/Zn concentration ratio was significantly affected by both the Z-score and the HDS-R score in the severe atrophy subgroup, with no multicollinearity of these two explanatory variables.

DISCUSSION

In the present study, we found that the serum Cu/Zn ratio was significantly higher in patients with AD, although the serum concentrations of Cu and Zn did not differ compared with the control group. Serum concentrations of Cu and Zn have previously been investigated in patients with AD, but no conclusive changes have been detected. Thus, the possibility of use of these elements for evaluating the pathophysiology of AD remains unclear. For example, Koç et al. and Molaschi et al. found that serum concentrations of Cu and Zn were decreased in AD patients[5)6)], while Molina et al. reported that they did not differ significantly between patients with AD and healthy participants[7)]. Baum et al. reported that serum Cu concentration were similar in patients with AD and healthy subjects, but serum Zn concentration were lower in AD patients[8)].

In an earlier exploratory evaluation of serum Cu and Zn concentrations based on previous data[5)-8)], we found that the serum Cu/Zn ratio was higher in patients with AD. The serum Cu/Zn ratio has recently gained attention as a biomarker for pathological conditions of oxidative stress[9)]; however, its usefulness in evaluating the pathophysiology of AD, which involves oxidative stress, has not been assessed.

Furthermore, factors that could affect concentrations of trace elements (e.g, hepatic, renal, metabolic, and gastrointestinal diseases, age, gender composition, and nutritional status) were not accounted for in previous studies[3)-8)]. Notably however, these potential biases were minimized since there was none of these factors between groups or subgroups in the present study.

This study investigated the relationship between AD pathophysiology and serum levels of Cu and Zn. Amyloid beta (Aβ) accumulates in the brain in patients with AD. Cu which binds to Aβ with Zn has an unstable electric charge and is particularly prone to oxidation and reduction, generating reactive oxygen species (ROS)[17)]. Excess generation of ROS results in oxidative stress which in turn contributes to neuronal loss in the brains of patients with AD. The antioxidant enzymes, metallothionein and superoxide dismutase (SOD) are induced to counteract oxidative stress. Metallothionein promotes the extracellular transport of Cu[18)]. SOD binds to Cu, which plays an antioxidant role in this enzyme, and to Zn, which maintains its structure[19)] A lack of intracellular Zn thus weakens the structure of SOD weakens, and SOD therefore promotes Zn uptake into the cell[20)]. This mechanism could contribute to increasing the serum Cu/Zn ratio during oxidative stress, as part of the pathology of AD.

We observed a positive correlation between the degree of medial temporal lobe atrophy and the serum Cu/Zn ratio in the AD group. The medial temporal lobe, a typical site of AD

lesions, contains decreased level of Cu and increased level of Zn[21)22)]. This reverse relationship of the Cu/Zn ratio between the serum and the lesion site may be partially explained by the tissue destructive release of Cu to the serum. However, there is currently no explanation for it, and this relationship requires further research.

The association between the degree of medial temporal lobe atrophy and the severity of AD assessed by HDS-R and MMSE was reported in a previous study[23)] and confirmed in the present study; however, we could not demonstrate the significant correlation between the severity of AD and the serum Cu/Zn ratio. We then performed a subgroup analysis of patients with AD according to the severity of medial temporal lobe atrophy. We found that the serum Cu/Zn ratio was higher in patients with severe medial temporal lobe atrophy and that the ratio was negatively correlated with the HDS-R score. Multiple regression analysis of the data for the severe atrophy subgroup showed that the severity of atrophy in the medial temporal lobe and the severity of AD assessed by the HDS-R independently affected the serum Cu/Zn ratio. Although the utility of the serum Cu/Zn ratio may be limited to patients with severe medial temporal lobe atrophy, this ratio may be an indicator of the pathophysiology of AD, including cognitive deficit and brain atrophy. Alternatively, the lack of a correlation between the Cu/Zn ratio and the MMSE may be explained by the lower score allocution to memory, which is most affected in AD, in the MMSE, compared with the HDS-R (13 points vs. 18 points)[24)].

The present study had several limitations. Our AD patients were clinically diagnosed to probable AD, however none of them were autopsied to definite AD. Since approximately 22% of cases of dementia are thought to be caused by multiple pathologies[25)], it is possible that the participants in the present study included individuals with both chronic ischemic stroke and AD. Further prospective studies are therefore required to evaluate longitudinal changes in the serum Cu/Zn ratio and cognitive function. In addition, the early diagnosis of mild cognitive impairment (MCI) has been attracting increasing attention. Approximately 20% of cases of MCI develop into dementia and 8.3% to AD[26)]. Further studies including patients with MCI patients may thus be beneficial for understanding the association between the serum Cu/Zn ratio and the pathophysiology of AD.

CONCLUSION

The present study suggests that the serum Cu/Zn ratio could be a candidate indicator for evaluating the pathophysiology of AD.

REFERENCES

1) Ikeda S, Mimura M, Ikeda M, Wada-Isoe K, Azuma M, Inoue S, et al. Economic burden of Alzheimer's disease dementia in Japan. J Alzheimers Dis. 2021; 81: 309–19.
2) Aliasgharpour M, Farzami MR. Trace elements in human nutrition: A review. Int J Med Invest. 2013; 2: 115–28.
3) Osredkar J, Sustar N. Copper and zinc, biological role and significance of copper/zinc imbalance. J Clinic Toxicol. 2011; S3: 001. doi:10.4172/2161-0495.S3-001.
4) Cicero CE, Mostile G, Vasta R, Rapisarda V, Signorelli SS, Ferrante M, et al. Metals and neurodegenerative diseases. A systematic review. Environ Res. 2017; 159: 82–94.
5) Koç ER, İlhan A, Aytürk Z, Acar B, Gürler M, Altuntaş A, et al. A comparison of hair and serum trace elements in patients with Alzheimer disease and healthy participants. Turk J Med Sci. 2015; 45: 1034–9.

6) Molaschi M, Ponzetto M, Bertacna B, Berrino E, Ferrario E. Determination of selected trace elements in patients affected by dementia. Arch Gerontol Geriatr. 1996; 22 suppl 1: 39–42.
7) Molina JA, Jiménez-Jiménez FJ, Aguilar MV, Meseguer I, Mateos-Vega CJ, González-Muñoz MJ, et al. Cerebrospinal fluid levels of transition metals in patients with Alzheimer's disease. J Neural Transm. 1998; 105: 479–88.
8) Baum L, Chan IHS, Cheung SK-K, Goggins WB, Mok V, Lam L, et al. Serum zinc is decreased in Alzheimer's disease and serum arsenic correlates positively with cognitive ability. Biometals. 2010; 23: 173–9.
9) Malavolta M, Piacenza F, Basso A, Giacconi R, Costarelli L, Mocchegiani E. Serum copper to zinc ratio: Relationship with aging and health status. Mech Ageing Dev. 2015; 151: 93–100.
10) McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services task force on Alzheimer's disease. Neurology. 1984; 34: 939–44.
11) Imai Y, Hasegawa K. The revised Hasegawa's dementia scale (HDS-R) -evaluation of its usefulness as a screening test for dementia. J. Hong Kong Coll Psychiatr. 1994; 4: 20–4.
12) Creavin ST, Wisniewski S, Noel-Storr AH, Trevelyan CM, Hampton T, Rayment D, et al. Mini-Mental State Examination (MMSE) for the detection of dementia in clinically unevaluated people aged 65 and over in community and primary care populations. Cochrane Database Syst Rev. 2016; 2016(1): CD011145. doi:10.1002/14651858.CD011145.pub2.
13) Abe A, Yamashita S, Noma A. Sensitive, direct colorimetric assay for copper in serum. Clin Chem. 1989; 35: 552–4.
14) Meret S, Henkin RI. Simultaneous direct estimation by atomic absorption spectrophotometry of copper and zinc in serum, urine, and cerebrospinal fluid. Clin Chem. 1971; 17: 369–73.
15) Matsuda H, Mizumura S, Nemoto K, Yamashita F, Imabayashi E, Sato N, et al. Automatic voxel-based morphometry of structural MRI by SPM8 plus diffeomorphic anatomic registration through exponentiated lie algebra improves the diagnosis of probable Alzheimer disease. AJNR Am J Neuroradiol. 2012; 33: 1109–14.
16) Rao YL, Ganaraja B, Murlimanju BV, Joy T, Krishnamurthy A, Agrawal A. Hippocampus and its involvement in Alzheimer's disease: a review. 3 Biotech. 2022; 12: 55. doi:10.1007/s13205-022-03123-4.
17) Cheignon C, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C, Collin F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. Redox Biol. 2018; 14: 450–64.
18) Gudekar N, Shanbhag V, Wang Y, Ralle M, Weisman GA, Petris MJ. Metallothioneins regulate ATP7A trafficking and control cell viability during copper deficiency and excess. Sci Rep. 2020; 10: 7856. doi:10.1038/s41598-020-64521-3.
19) Manieri TM, Sensi SL, Squitti R, Cerchiaro G. Structural effects of stabilization and complexation of a zinc-deficient superoxide dismutase. Heliyon 2021; 7: e06100. doi:10.1016/j.heliyon.2021.e06100.
20) Homma K, Fujisawa T, Tsuburaya N, Yamaguchi N, Kadowaki H, Takeda K, et al. SOD1 as a molecular switch for initiating the homeostatic ER stress response under zinc deficiency. Mol cell. 2013; 52: 75–86.
21) Moynier F, Borgne ML, Lahoud E, Mahan B, Mouton-Liger F, Hugon J, et al. Copper and zinc isotopic excursions in the human brain affected by Alzheimer's disease. Alzheimers Dement (Amst). 2020; 19: 12: e12112. doi:10.1002/dad2.12112.
22) Xu J, Church SJ, Patassini S, Begley P, Waldvogel HJ, Curtis MA, et al. Evidence for widespread, severe brain copper deficiency in Alzheimer's dementia. Metallomics. 2017; 9: 1106–19.
23) Yamashita K, Kuwashiro T, Ishikawa K, Furuya K, Harada S, Shin S, et al. Identification of predictors for mini-mental state examination and revised Hasegawa's Dementia Scale scores using MR-based brain morphometry. Eur J Radiol Open. 2021; 8: 100359.
24) Kim KW, Lee DY, Jhoo JH, Youn JC, Suh YJ, Jun YH, et al. Diagnostic accuracy of mini-mental status examination and revised Hasegawa

dementia scale for Alzheimer's Disease. Dement Geriatr Cogn Disord. 2005: 19; 324–30.
25) Mitchell AJ, Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia-meta-analysis of 41 robust inception cohort studies. Acta Psychiatr Scand. 2009: 119; 252–65.
26) Ishikawa T, Ikeda M. Mild cognitive impairment in a population-based epidemiological study. Psycogeriatrics. 2007; 7: 104–8.