Serum Zinc Levels and in Vivo Beta-Amyloid Deposition in the Human Brain

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Abstract

Background: Despite the known associations between zinc levels and Alzheimer's disease (AD) dementia and related cognitive impairment, the underlying neuropathological links remain poorly understood. We tested the hypothesis that serum zinc level is associated with cerebral beta-amyloid protein (Ab) deposition. Additionally, we explored associations between serum zinc levels and other AD pathologies [i.e., tau deposition and AD-signature cerebral glucose metabolism (AD-CM)] and white matter hyperintensities (WMHs), which are measures of cerebrovascular injury.

Methods: A total of 241 cognitively normal older adults between 55 and 90 years of age were enrolled. All the participants underwent comprehensive clinical assessments, serum zinc level measurement, and multimodal brain imaging, including Pittsburgh compound B-positron emission tomography (PET), AV-1451 PET, fluorodeoxyglucose (FDG)-PET, and magnetic resonance imaging. Zinc levels were stratified into three categories: < 80 µg/dL (low), 80 to 90 µg/dL (medium), and > 90 µg/dL (high).

Results: A low serum zinc level was significantly associated with increased Ab retention. In addition, apolipoprotein E ε4 allele (APOE4) status moderated the association: the relationship between low zinc level and Ab retention was significant only in APOE4 carriers. Although a low zinc level appeared to reduce AD-CM, the relationship became insignificant on sensitivity analysis including only individuals with no nutritional deficiency. The serum zinc level was associated with neither tau deposition nor the WMH volume.

Conclusions: Our findings suggest that decreased serum zinc levels are associated with elevation of brain amyloid deposition. In terms of AD prevention, more attention needs to be paid to the role of zinc.

Background

Zinc is the most abundant trace metal in the brain.[1] Disruption of zinc homeostasis may play a critical role in the pathogenesis of Alzheimer's disease (AD).[1–3] Many human studies have found that serum zinc levels were decreased in AD dementia compared to healthy controls.[4–8] Low serum zinc levels were also associated with rapid progression of AD dementia[2] and poorer cognitive performance.[9]

Despite these associations, the pathological mechanisms in play remain unclear. Two preclinical studies using an AD mouse model revealed that brain zinc bound to beta-amyloid protein (Aβ) plaques and that the levels remained high therein,[10] and that zinc treatment increased amyloid precursor protein (APP) expression, enhanced amyloidogenic APP cleavage and Aβ deposition, and impaired spatial learning and memory.[11] A postmortem brain study also indicated that brain zinc accumulation was a prominent feature of AD, linked to brain Aβ accumulation and dementia severity.[3] Serum zinc concentrations from 12 sisters who died in the Nun Study, determined approximately 1 year before death, also showed inverse correlations with senile plaque counts in the brain.[12] However, as of yet, no study has investigated the relationship between serum zinc levels and Aβ deposition in the living human brain. Thus, we tested the hypothesis that serum zinc level is associated with brain Aβ deposition in cognitively normal (CN) older

adults. Additionally, we explored the associations between serum zinc level and other AD pathologies (i.e., tau deposition and AD-signature neurodegeneration) and white matter hyperintensities (WMHs), which are measures of cerebrovascular injury.

Methods

Participants

This study was part of the Korean Brain Aging Study for Early Diagnosis and Prediction of Alzheimer's Disease (KBASE), which is an ongoing prospective cohort study.[13] As of February 2017, a total of 241 CN older adults between 55 and 90 years of age were enrolled. The CN group consisted of participants with a Clinical Dementia Rating (CDR)[14] score of 0 and no diagnosis of mild cognitive disorder or dementia. The exclusion criteria were as follows: 1) A major psychiatric illness; 2) a significant neurological (e.g., cerebrovascular) disease or any medical condition that could affect mental function; 3) contra-indications for magnetic resonance imaging (MRI) (e.g., a pacemaker or claustrophobia); 4) illiteracy; 5) the presence of significant visual/hearing difficulties and/or severe communication or behavioral problems that would render clinical examinations or brain scans difficult; and, 6) use of an investigational drug. Exclusion criteria was sought by research clinicians who examined laboratory data, MRI results, clinical data collected by trained nurses during systematic interviews of participants, and reliable informants to whom we spoke during screening. More detailed information on recruitment was presented in our previous report.[13]

Clinical Assessments

All participants underwent comprehensive clinical and neuropsychological assessments administered by trained psychiatrists and neuropsychologists based on the KBASE assessment protocol.[13] This incorporates the Korean version of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological battery.[15–17] Vascular risk factors (e.g., hypertension, diabetes mellitus, dyslipidemia, coronary heart disease, transient ischemic attack, and stroke) were assessed based on data collected by trained nurses during systematic interviews of participants and their family members. A vascular risk score (VRS) was calculated based on the number of vascular risk factors present and reported as a percentage.[18] Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. The Geriatric Depression Scale (GDS) was used to measure the severity of depressive symptoms.[19, 20] Annual income was evaluated and categorized into three groups [below the minimum cost of living (MCL), more than the MCL but below twice the MCL, and twice the MCL or more] (<http://www.law.go.kr>). The MCL was determined according to the administrative data published by the Ministry of Health and Welfare, Republic of Korea in November 2012. The MCL was 572,168 Korean Won (KRW) [equivalent to 507.9 US dollars (USD)] per month for single-person households with an additional 286,840 KRW (equivalent to 254.6 USD) per month for each additional housemate. To ensure that the information was accurate, reliable informants were also interviewed.

Measurement of serum zinc levels and other blood biomarkers

After an overnight fasting, blood samples were obtained via venipuncture in the morning (8–9 a.m.). We measured serum zinc and copper levels using an inductively coupled plasma-mass spectrometer (model 820-MS; Bruker, Australia). We also measured serum copper, calcium, iron, transferrin, and ceruloplasmin levels because they could confound the relationship between zinc levels and brain changes.[21] We measured blood hemoglobin, albumin, and total cholesterol levels to evaluate anemia and nutritional deficiency. Calcium, iron, albumin, and total cholesterol levels were measured using a colorimetric method (ADVIA 1800 Auto analyzer, Siemens, USA). Transferrin and ceruloplasmin were measured employing immunoturbidimetric assays (Cobas Integra 800, Roche Diagnostics). Albumin levels were automatically determined (Advia 1800; Siemens, USA). Hemoglobin levels were determined by flow cytometry (Advia 2120i; Siemens, USA). Genomic DNA was extracted from whole blood and apolipoprotein E (apoE) genotyping performed as previously described.[22] ApoE ϵ 4 allele (APOE4)-positivity was defined as the presence of at least one ϵ 4 allele.

Measurement of cerebral A β deposition

All participants underwent contemporaneous three-dimensional (3D) [^{11}C] Pittsburgh compound B (PiB)-positron emission tomography (PET) and 3D T1-weighted MRI scanning using a 3.0-T Biograph mMR (PET-MR) platform (Siemens, USA), in accordance with the manufacturer's guidelines. The PiB-PET imaging acquisition and preprocessing details were described previously.[23] An automatic anatomical labeling algorithm and a region-combining method[24] were applied to determine regions of interest (ROIs) for characterization of PiB retention levels in the frontal, lateral parietal, posterior cingulate-precuneus, and lateral temporal regions. Standardized uptake value ratio (SUVR) values for each ROI were calculated by dividing the mean value for all voxels within each ROI by the mean cerebellar uptake value in the same image. A global cortical ROI consisting of the four ROIs was defined and a global A β retention value was generated by dividing the mean value for all voxels of the global cortical ROI by the mean cerebellar uptake value in the same image.[24, 25]

Measurement of cerebral tau deposition

A subset of subjects ($n = 58$) underwent [^{18}F] AV-1451 PET scans using a Biograph True Point 40 PET/CT platform (Siemens, USA), in accordance with the manufacturer's guidelines. Although all the other neuroimaging scans were performed during the baseline visit, AV-1451 PET imaging was performed at an average of 2.55 (standard deviation = 0.26) years after that visit. The details of AV-1451 PET imaging acquisition and preprocessing have been described previously.[23] To estimate cerebral tau deposition, we quantified the AV-1451 SUVR of an a priori ROI of the "AD-signature region" of tau accumulation, which was a size-weighted average of the partial volume-corrected uptakes by the entorhinal, amygdala, parahippocampal, fusiform, inferior temporal, and middle temporal ROIs, in line with a published method.[26] The AV-1451 SUVR of this ROI served as the outcome variable for cerebral tau deposition.

Measurement of AD-signature neurodegeneration

All participants underwent [18F] fluorodeoxyglucose (FDG)-PET imaging using the above-described PET-MR platform; the details of FDG-PET image acquisition and preprocessing have been described previously.[23] AD-signature FDG ROIs that are sensitive to the changes associated with AD, such as the angular gyri, posterior cingulate cortex, and inferior temporal gyri,[27] were determined. AD-signature cerebral glucose metabolism (AD-CM) was defined as the voxel-weighted mean SUVR extracted from the AD-signature FDG ROIs.

Measurement of WMH

All participants underwent MRI including T1-weighted images and fluid-attenuated inversion recovery (FLAIR) imaging using the abovementioned 3.0-T PET-MR platform. WMH volume was measured using a validated automatic procedure that has been previously reported.[28] Briefly, the procedure consists of 11 steps: spatial co-registration of T1 and FLAIR images, fusion of T1 and FLAIR images, segmentation of T1 images, acquisition of transformation parameters, deformation and acquisition of the white matter mask, acquisition of FLAIR within the white matter mask, intensity normalization of the masked FLAIR, nomination of candidate WMH with a designated threshold, creation of a junction map, and elimination of the junction. There were two modifications in the current processing procedure relative to that used in the original study: (a) an optimal threshold of 70 was applied, as it was more suitable for our data than the threshold of 65 that was used in the original study; and, (b) given that individuals with acute cerebral infarcts were not enrolled in our sample, we did not use diffusion-weighted imaging in the current automated procedure. Using the final WMH candidate image, WMH volume was extracted in the native space in each subject.

Statistical analysis

To examine the relationships between the serum zinc level and neuroimaging biomarkers, multiple linear regression analyses were performed as appropriate. The zinc level, which was an independent variable in each analysis, was entered as a stratified categorical variable (three categories: low < 80 µg/dL, medium 80 to 90 µg/dL, and high > 90 µg/dL) by reference to the criteria for zinc deficiency (deficient < 60 µg/dL, marginal deficiency ≥ 60 to < 80 µg/dL, and normal ≥ 80 µg/dL).[29] On statistical analysis, deficiency and marginal deficiency were combined into the low category, because only one participant exhibited a deficiency. Within the normal zinc range, the median value (i.e., 90 µg/dL) served as the cutoff between the low-normal (medium) and high-normal (high) levels. To analyze the associations between serum zinc levels and neuroimaging biomarkers, two models were used for stepwise control of potential confounders. The first model did not include any covariate and the second model included all potential covariates (i.e., age, sex, educational level, APOE4-positivity status, the VRS, the BMI, the GDS score, annual income status, and albumin, copper, calcium, iron, transferrin, and ceruloplasmin levels) that might confound the relationship between the zinc level and brain change.[21] On multiple linear regression analyses, global Aβ retention and WMH values were subjected to natural log-transformations to achieve normal distributions. In all analyses, a high zinc level served as the reference (i.e., high zinc vs.

medium zinc, or high zinc vs. low zinc). Sensitivity analyses were performed for the participants with no nutritional deficiencies (i.e., serum albumin < 3.5 g/dL and total cholesterol < 150 mg/dL)[30, 31] to reduce the influence of a general nutritional deficiency on the association between serum zinc level and brain changes. To explore the influence of age, sex, APOE4-positivity, VRS, and the copper, calcium, and iron levels on the associations between serum zinc levels and neuroimaging biomarkers that were significant in the analyses described above, the regression analyses were repeated but now including two-way interaction terms between serum zinc levels and the biomarkers as additional independent variables. All statistical analyses were performed with the aid of IBM SPSS Statistics software (version 27, IBM Corp., Armonk, NY, USA).

Results

Participant characteristics

The demographic and clinical characteristics of all participants are presented in Table 1. Of the total of 241 participants, 70 had low zinc levels, 80 had medium zinc levels, and 91 had high zinc levels.

Association of serum zinc level with cerebral Ab deposition

Low serum zinc was associated with significantly increased global Ab retention compared to the high serum zinc (Table 2 and Figure 1). Sensitivity analysis of participants with no nutritional deficiencies yielded similar results (Table 3).

Association of the zinc level with other brain pathologies

No differences in terms of tau deposition or WMH were observed between the serum zinc categories (Tables 2 and 3). A reduced AD-CM was related to a low serum zinc level (Table 2), but the relationship became insignificant on sensitivity analysis of only individuals with no nutritional deficiency (Table 3).

Moderation of the association between the serum zinc level and cerebral Ab deposition

The serum zinc x APOE4-positivity interaction was significant in terms of Ab retention, indicating that APOE4-positivity moderates the association between the serum zinc level and cerebral Ab deposition (Table 4). Further subgroup analyses showed that low serum zinc was significantly associated with higher Ab deposition in the APOE4-positive but not -negative subgroup (Table 5 and Figure 1). The interactions between the serum zinc level and other variables including sex, the VRS, and copper, calcium, and iron levels, were not significant (Table 4).

Discussion

In older individuals lacking cognitive impairment, low serum zinc was associated with increased in vivo global A β retention, supporting the hypothesis that low serum zinc is associated with high brain amyloid deposition, in line with the inverse correlation between serum zinc levels determined about 1 year before

death and the post-mortem senile plaque counts in 12 individuals of the Nun study.[12] Many clinical studies have also reported associations between low serum zinc levels and clinical AD dementia or poor cognitive performance. In contrast, a postmortem study revealed that higher brain zinc accumulation was associated with increased brain A β deposition and dementia severity.[3] As zinc bound to A β plaques and remained high in such plaques in an AD mouse model,[10] a low serum zinc level may reflect higher A β deposition in the brain, in turn increasing zinc accumulation and sequestration of zinc in the brain, depleting zinc in other body compartments such as blood.[12]

We also found that APOE4 status moderated the relationship between the serum zinc level and amyloid deposition. We found a significant negative association between the zinc level and A β deposition in participants with APOE4, but not those without APOE4. This may reflect associative interactions between zinc, the apoE4 isoform, and A β deposition.[32] ApoE4 binds to A β and facilitates A β fibrillation more easily than do the apoE2 and apoE3 isoforms.[33, 34] Additionally, as the sulfhydryl groups of cysteine residues are responsible for zinc-binding,[35, 36] the arginine substitutions in apoE4 restrict its ability to control zinc homeostasis and zinc-dependent molecular changes in the AD brain.[37]

We additionally found a significant association between a low serum zinc level and decreased AD-CM. In contrast, we found no association between the serum zinc level and either tau deposition or WMH, indicating that zinc-related hypometabolism is possibly not mediated by brain tau deposition or cerebrovascular injury. The interaction of zinc with A β within A β plaques in the AD brain may trigger a neuronal zinc imbalance at the glutamatergic synapse level, further exacerbating synaptic dysfunction and the associated cerebral hypometabolism.[1, 8, 38, 39] Another possible explanation for the association between a low serum zinc level and brain hypometabolism is that the association may be cofounded by nutritional status; a nutritional deficiency is associated with both low serum zinc levels and reduced brain metabolism.[40] Sensitivity analysis of only participants lacking nutritional deficiencies revealed that the association between the serum zinc level and AD-CM was no longer significant, supporting the above explanation.

Limitations

To the best of our knowledge, this study is the first to show an association between the serum zinc level and brain A β accumulation in living humans. Even after controlling for all potential confounders, the findings did not change. The results were confirmed by sensitivity analysis performed after excluding participants with nutritional deficiencies. However, our study had a couple of limitations. First, as this was a cross-sectional work, causal relationships cannot be inferred. Long-term prospective studies are needed. Second, tau PET was performed at an average of 2.55 years (standard deviation 0.26 years) after the baseline visit; the other neuroimaging scans were performed at baseline. This temporal gap may have influenced the association between zinc and tau. When we controlled for the temporal gap as an additional covariate, however, the results did not change. In addition, only a subset of participants ($n = 58$) underwent tau PET, whereas all participants underwent all other imaging modalities. The lower tau PET

sample size may have decreased the statistical power and thus contributed to the null result for the relationship between zinc level and tau deposition. A study with a larger sample size is required.

Conclusion

The present findings suggest that a decreased serum zinc level are associated with elevation of brain amyloid deposition, particularly in APOE4 carriers. In terms of AD prevention, more attention needs to be paid to the role of zinc.

Declarations

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Author Contributions

JWK and DYL conceived and designed the study. MSB, DY, JHL, MJK, GJ, J-YL, KMK, C-HS, Y-SL, YKK, and DYL were involved in acquisition, or analysis and interpretation of the data and helped to draft the manuscript. JWK, MSB, DY, JHL, and DYL were major contributors in writing the manuscript and critically revising the manuscript for intellectual content. DYL served as principal investigator and supervised the study. All authors read and approved the final manuscript.

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Availability of data and materials

The data of the current study are not freely accessible because the Institutional Review Board (IRB) of Seoul National University Hospital prohibits public data-sharing for privacy reasons. However, the data may be available from the independent data-sharing committee of the KBASE research group on reasonable request, after approval by the IRB. Requests for data access can be submitted to the administrative coordinator of the KBASE group by e-mail (kbasecohort@gmail.com); the coordinator is independent of the authors.

Ethics approval and consent to participate

This study protocol was approved by the Institutional Review Boards of Seoul National University Hospital (SNUH) (C-1401-027-547) and Seoul Metropolitan Government-Seoul National University (SMG-SNU) Boramae Medical Center (26-2015-60), Seoul, South Korea, and was conducted in accordance with the recommendations in the current version of the Declaration of Helsinki. The subjects or their legal representatives gave written informed consent.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 1-5 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Bar charts displaying stratified serum zinc levels and global A β retentions in cognitively normal older participants. (A) overall and (B) by subgroup (APOE4-positivity and -negative). Footnotes: Error bars indicate standard errors. Multiple linear regression analyses were performed after adjusting for all confounding factors.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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- [STROBEchecklistcrosssectional.docx](#)
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