The Role of Iron in Alzheimer's Disease: A Promising Biomarker

Kristin Tsai[1,2], Sebastian Crespo[1,3], Sumuga Swaminathan[1,4]
[1] *International Youth Neuroscience Association,* [2] *Carnegie Vanguard High School,* [3] *Mark T. Sheehan High School,* [4] *Lake Norman High School*

Abstract

Alzheimer's disease (AD) is a neurodegenerative condition, often associated with aging where the exact biomarkers are still being studied. Amyloid plaques and tau aggregations are commonly associated biomarkers in Alzheimer's Disease (AD), as blood based biomarkers are an upcoming approach to detect AD biomarkers (Hampel et al., 2023). In previous studies, a relation between iron and AD have been investigated, where high levels of ferritin were associated with cognitive impairment (Liu et al., 2018). Furthermore, in vitro studies have been conducted and discovered that high brain iron causes aβ aggregation, which implies that iron may play a bigger role in AD (Liu et al., 2018). In the cellular perspective, ferroptosis, a newly discovered process of non-apoptotic cell death, is related to elevated levels of iron in the brain and depleted glutathione as a marker, which may provide a substantial lead in the cause of neuronal cell death in Alzheimer's and other neurodegenerative diseases (Li et al., 2020. Sun 2018). Previous studies of ferritin in CSF corresponding to Alzheimer's disease have been done, but the use of blood biomarkers and longitudinal studies for iron as a biomarker need to be investigated (Ayton et al., 2015). The purpose of this study was to explore iron as a promising biomarker in Alzheimer's. An experiment was conducted using blood samples from 2 groups of people, non AD and AD groups, where levels of ferritin and plasma related biomarkers were tested. To further confirm our groups, we utilized postmortem tissue to detect and iron accumulation in the brain. Our findings suggest that iron (serum ferritin) was consistently associated in high levels with Alzheimer patient brains, while the control group had a significantly lesser accumulation, suggesting iron to be a valid AD biomarker.

Keywords: blood biomarkers, ferroptosis, Alzheimer's disease, brain iron, neurodegeneration

Introduction

Alzheimer's is a progressive dementia that begins with memory problems at an early stage and can eventually progress to a severe stage in which the patient is completely dependent on care (BrainFacts, 97). It is believed that family genetics may play a big role in developing AD. However, AD, being the most common neurodegenerative disorder, often requires the search for biomarkers in the hope of diagnosing the patient for the disorder. Blood biomarkers are measurable indicators found in the blood that provide information about biological processes, conditions, or diseases in the body. In the context of AD, blood biomarkers may reflect changes in the brain associated with AD pathology, such as the accumulation of aβ plaques, tau protein abnormalities, neuronal damage, etc (Hannson, 2022). Iron is a substance that can potentially influence biomarkers associated with AD. For example, Iron has been shown to promote the aggregation and accumulation of aβ, Iron dysregulation can also impact tau protein metabolism, and abnormal iron metabolism synthesizes hydroxyl radicals through the Fenton reaction,

triggering oxidative stress, and damaging cell structures (Liu, Fan, Yang, Wang and Guo, 2018). All of these factors may contribute to AD. Aβ aggregation/accumulation and tau aggregation are other pathological features of AD, and biomarkers of oxidative stress may be elevated in response to increased iron levels and oxidative damage in AD. Glutathione (GSH) plays a role in balancing oxidative stress. There are significantly less GSH levels in the hippocampus in AD patients, indicating that elevated oxidative stress relates with AD. Iron dysregulation for example, is a form of Ferroptosis, which is a form of cell death characterized by iron-dependent lipid peroxidation and accumulation of reactive oxygen species (ROS). Ferritin, a storage protein for iron, which has elevated levels in CSF of AD patients (Ayton, 2022) providing a valuable point of expansion for detection. Alzheimer's is marked by neuronal and glial apoptosis (Shimohama et al., 2000). The findings of brain iron accumulation can imply ferroptosis occurring, especially in the hippocampus, the region most affected by AD, could lead to the degeneration of memory neurons resulting in dementia. The objective of this proposal is to investigate whether iron dysregulation, identified through ferritin in blood, provides a strong association/biomarker for AD due to the linkage to ferroptosis, a neuronal cell death associated with AD. Using the AT(N) framework, we will test for amyloid beta, tau, nfl levels and compare with ferritin levels to validate this biomarker.

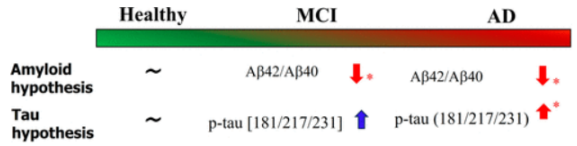

Fig. 2
Existing plasma-based biomarkers to screen MCI and AD patients. Tests with statistically significant outcomes are marked by an asterisk (∗). Significantly lower value of Aβ42/40 ratio was reported in amnestic-MCI patients compared to healthy subjects. (2) P-tau 217 refers to phosphorylation of threonine residues at 217 amino acid position. In AD-affected brains, tau protein is present in the form of abnormal neurofibrillary tangles. Autopsy studies from AD-affected brains display a direct correlation between disease severity and tangle deposition. (Mandal, 2023)

Table 1 | The eight AT(N) profiles

Table 1 | **The eight AT(N) profiles**

Profile	Biomarker category	AD continuum?
A–T–N–	Normal AD biomarkers	No
A+T–N–	AD pathological change	Yes
A+T+N–	AD	
A+T+N+		
A+T–N+	AD and concomitant suspected non-AD pathological change	
A–T+N–	Non-AD pathological change	No
A–T–N+		
A–T+N+		

A, amyloid-β; AD, Alzheimer disease; N, neurodegeneration; T, tau. TABLE 1 reprinted with permission from REF.[4], Elsevier.

(Hampel, 2021)

Methodology

Using a multi-technological approach, the serum ferritin levels, tau (plasma p-tau), plasma nfl, and plasma aβ 42/40 levels of 2 groups of individuals, aged 50-80 (both n=150) will be obtained. The first group (G1, control) will be individuals without Alzhimers and a healthy record of no iron related disease and the second group (G2), will be individuals with Alzhimers (A+, T+) by random selection of consenting adults through medical paperwork at local hospitals. PET/MRI scans will be utilized to confirm AD for G2. Prior to the testing, all individuals will have a low iron diet for a week, and fast for at least 8 hours. The first part of this experiment will involve drawing the blood sample and centrifuge for plasma of both groups to screen for plasma aβ, plasma p-tau 217, plasma nfl, and ferritin. We will do the same process every 3 months for a year to document changes.The Invitrogen Amyloid beta 42 Human ELISA Kit, Ultrasensitive and Invitrogen Amyloid beta 40 Human ELISA Kit (Thermo Fisher) were used for plasma aβ 42/40 ratio detection. The Human p-tau217 (Phospho Tau 217) ELISA Kit (Assay Genie) was used for plasma p-tau 217 detection. The Human Neurofilament-Light Chain (NFL) ELISA Kit (MBS) was utilized, and Invitrogen Ferritin Human ELISA Kit (Thermo Fisher) was used. All of these assays were 96 well plates, utilized a wash buffer, and HRP diluents. In G1, mean plasma aβ 42/40 ratios were around ≥0.170, suggesting low presence of AD, compared to G2, with mean levels <0.150. For plasma p-tau217 concentration levels and plasma nfl levels, G1 had a significantly lower pg/ml compared to G2. The serum ferritin levels were found to be elevated in G2 (average around 300 ng/L) rather than G1 after screening for A, T, N biomarkers. To further confirm iron's role in Alzheimer's, 50 brain samples from the experimental group (verified AD) and 50 brain samples from the control group (healthy subjects without an anemic history) (age at death 60-85 years) (Langkammer et al., 2011). Following the brain extraction, iron concentrations were determined using inductively coupled plasma mass spectrometry in prespecified regions of white and gray matter proximal to the hippocampus as well as the hippocampus. (Langkammer et al., 2011)

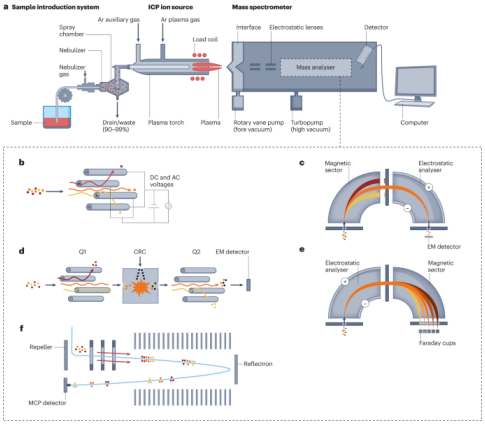

Figure 3: Illustrates the process of inductively coupled plasma mass spectrometry (ICP-MS) (Acker et al., 2023)

Comparison of plasma ptau 217 levels in non AD vs. AD samples

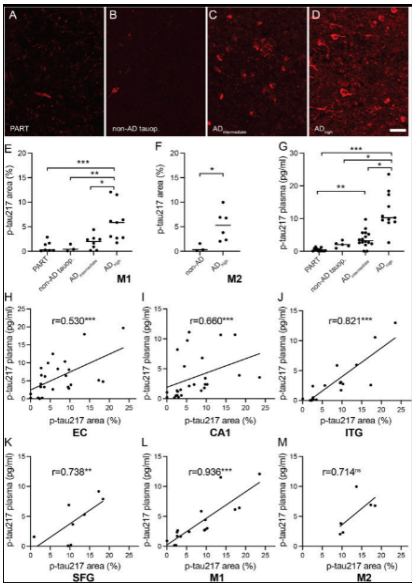

Fig. 4 *Analysis of p-tau217 area fraction, tangles and GVB in Cohort 1. Picture in (**A**) illustrates hippocampus and the CA1 area where the pictures (red dotted squares) were captured. Images in (**B**–**C**) represents pictures captured from non-demented individuals (NC) without amyloid beta (NC−Aβ) (**B**), NC with amyloid beta (NC+Aβ) (**C**), Alzheimer's disease (AD) patients with moderate AD pathology (ADmod) (D) and AD patients with severe AD pathology (ADsev) (E). Scalebar = 40 µm. Graph in (**F**–**G**) illustrate the area fraction of p-tau217 (**F**), number of p-tau217 positive NFTs (**G**) and p-tau217 positive GVBs (**H**) in NC−Aβ, NC+Aβ, ADmod and ADsev All variables were significantly higher in ADsev compared to NC−Aβ, NC+Aβ. Data was analyzed using was analyzed using Kruskal Wallis Test corrected for Benjamini–Hochberg False discovery rate (FDR). Scatter plot in (**I**) show that number of tangles tends to increase along with GVB in ADmod, whereas the opposite pattern is seen in ADsev.. Data in (**I**) was analyzed using Spearman correlations test. Each point represents a mean of 3 pictures from 3 sections (in total 9) from each individual. * = p < 0.05, ** = p < 0.01, *** = p < 0.001*
(Wennstrom et al., 2022)

Discussion of Results

Due to the scope of iron and iron related dysfunctions visible to have on the progression on AD, it can be a biomarker utilized to diagnose and indicate the potential of developing AD. Our postmortem examination results indicated the highest amount of iron concentrations in the hippocampus, demonstrating a correlation between high ferritin levels and Alzheimer's. Furthermore, using blood biomarkers, individuals with AD were shown to have high plasma tau and nfl levels and low aβ levels, and the serum ferritin were found to be higher (above 300 µg/L). By multi approaches to confirm the presence AD condition in blood biomarkers, we now are able to suggest a strong validation for iron ferritin to be an AD biomarker. To eliminate any confounding variables, we eliminated anemic history patients from the study, as well as those with chronic inflammatory diseases, as high ferritin could be present in such individuals. Ferritin levels in the blood can also be related to inflammation in other regions (Garcia-Casal et al., 2021), which may not be a clear indicator of ferroptosis. In the future, gender related iron and AD studies could be implemented, to investigate the differing degrees of iron and AD. Through

using blood to detect for high ferritin and serum iron levels as well as the AT(N) framework to determine a direct association, we are able to receive a novel, multidimensional, convenient approach to further advance the study and diagnosing process of AD.The association between high ferritin and other blood biomarkers for AD demonstrate the strengthening evidence on the reliability of iron as a biomarker. Furthermore, using the same correlation, it can be noted that ferroptosis (increased levels of ferritin insinuating inflammation) plays a role in neuronal cell death, which could also make ferritin or ferroptosis related blood biomarkers valid for neurodegeneration, adding it onto the AT(N) framework (ATF(N)) framework. By relying on these technologies and existing frameworks of iron and AD relation, this will allow clinicians to detect people predisposed to Alzheimer's through ferritin levels and the AT(N) framework, further narrowing down potential alternative therapies (for example, if amyloid targeted therapies fail, then iron related treatments, like iron chelation could help), lighting a new perspective in NDD research.

Acknowledgements

We would like to thank our mentor, PhD candidate Xuelin Gu, for providing guidance throughout the development of this proposal. Through his efforts in assisting us in various parts of the methodology process as well as giving us professional feedback, we were able to construct a detailed research proposal.

References

[1]
Abnormal iron build-up in the brain linked to Alzheimer’s disease. (2022). Nature India. https://doi.org/10.1038/d44151-022-00102-9

[2]
Ayton, S., Faux, N., Bush, A. et al. Ferritin levels in the cerebrospinal fluid predict Alzheimer’s disease outcomes and are regulated by APOE. Nat Commun 6, 6760 (2015). https://doi.org/10.1038/ncomms7760

[3]
Ayton, S., Shorena Janelidze, Kalinowski, P., Palmqvist, S., Belaidi, A. A., Stomrud, E., Roberts, A., Roberts, B., Hansson, O., & Ashley Ian Bush. (2022). CSF ferritin in the clinicopathological progression of Alzheimer’s disease and associations with APOE and inflammation biomarkers. Journal of Neurology, Neurosurgery, and Psychiatry, jnnp-330052. https://doi.org/10.1136/jnnp-2022-330052

[4]
Cerasuolo, M., Irene Di Meo, Maria Chiara Auriemma, Trojsi, F., Maria Ida Maiorino, Cirillo, M., Esposito, F., Polito, R., Anna Maria Colangelo, Paolisso, G., Papa, M., & Maria Rosaria Rizzo. (2023). Iron and Ferroptosis More than a Suspect: Beyond the Most Common Mechanisms of Neurodegeneration for New Therapeutic Approaches to Cognitive Decline and Dementia. International Journal of Molecular Sciences, 24(11), 9637–9637. https://doi.org/10.3390/ijms24119637

[5]

Domenico, F., & Lanzillotta, C. (2022). Iron in Alzheimer's Disease: From Physiology to Disease Disabilities. ProQuest, 1248. https://doi.org/10.3390/biom12091248

[6]
Garcia-Casal, M. N., Pasricha, S. R., Martinez, R. X., Lopez-Perez, L., & Peña-Rosas, J. P. (2021). Serum or plasma ferritin concentration as an index of iron deficiency and overload. The Cochrane database of systematic reviews, 5(5), CD011817. https://doi.org/10.1002/14651858.CD011817.pub2

[7]
Gao, G., You, L., Zhang, J., Chang, Y. Z., & Yu, P. (2023). Brain Iron Metabolism, Redox Balance and Neurological Diseases. Antioxidants (Basel, Switzerland), 12(6), 1289. https://doi.org/10.3390/antiox12061289

[8]
Greenough, M. A., Lane, D. J. R., Balez, R., Anastacio, H. T. D., Zeng, Z., Ganio, K., McDevitt, C. A., Acevedo, K., Belaidi, A. A., Koistinaho, J., Ooi, L., Ayton, S., & Bush, A. I. (2022). Selective ferroptosis vulnerability due to familial Alzheimer's disease presenilin mutations. Cell Death & Differentiation, 1–14. https://doi.org/10.1038/s41418-022-01003-1

[9]
Ghaderi, S., Mohammadi, S., Nezhad, N. J., Karami, S., & Sayehmiri, F. (2024). Iron quantification in basal ganglia: quantitative susceptibility mapping as a potential biomarker for Alzheimer's disease - a systematic review and meta-analysis. Frontiers in neuroscience, 18, 1338891. https://doi.org/10.3389/fnins.2024.1338891

[10]
Hampel, Harald et al. (2023). Blood-based biomarkers for Alzheimer's disease: Current state and future use in a transformed global healthcare landscape. Neuron, Volume 111, Issue 18, 2781 - 2799

[11]
Hampel, H., Cummings, J., Blennow, K., Gao, P., Jack, C. R., & Vergallo, A. (2021). Developing the ATX(N) classification for use across the Alzheimer disease continuum. Nature Reviews Neurology, 17(9), 580–589. https://doi.org/10.1038/s41582-021-00520-w

[12]
Jakaria, Md., Belaidi, A. A., Bush, A. I., & Ayton, S. (2021). Ferroptosis as a mechanism of neurodegeneration in Alzheimer's disease. Journal of Neurochemistry, 159(5), 804–825. https://doi.org/10.1111/jnc.15519

[13]
Langkammer, C., Krebs, N., Goessler, W., Scheurer, E., Ebner, F., Yen, K., Fazekas, F., & Ropele, S. (2010). Quantitative MR imaging of brain iron: a postmortem validation study. Radiology, 257(2), 455–462. https://doi.org/10.1148/radiol.10100495

[14]
Li, J., Cao, F., Yin, H., Huang, Z., Lin, Z., Mao, N., Sun, B., & Wang, G. (2020). Ferroptosis: past, present and future. Cell Death & Disease, 11(2), 1–13. https://doi.org/10.1038/s41419-020-2298-2

[15]
Liu, J. L., Fan, Y. G., Yang, Z. S., Wang, Z. Y., & Guo, C. (2018). Iron and Alzheimer's Disease: From Pathogenesis to Therapeutic Implications. Frontiers in neuroscience, 12, 632. https://doi.org/10.3389/fnins.2018.00632

[16]
Mandal, P. K., Maroon, J. C., Garg, A., Narendra Kumar Arora, Bansal, R., Kaushik, A., Avantika Samkaria, Kumaran, G., & Arora, Y. (2023). Blood Biomarkers in Alzheimer's Disease. ACS Chemical Neuroscience, 14(22), 3975–3978. https://doi.org/10.1021/acschemneuro.3c00641

[17]
Ndayisaba, A., Kaindlstorfer, C., & Wenning, G. K. (2019). Iron in Neurodegeneration - Cause or Consequence?. Frontiers in neuroscience, 13, 180. https://doi.org/10.3389/fnins.2019.00180

[18]
Sun, Y., Zheng, Y., Wang, C., & Liu, Y. (2018). Glutathione depletion induces ferroptosis autophagy, and premature cell senescence in retinal pigment epithelial cells. Cell Death & Disease, 9(7). https://doi.org/10.1038/s41419-018-0794-4

[19]
Tran, D., DiGiacomo, P., Born, D. E., Georgiadis, M., & Zeineh, M. (2022). Iron and Alzheimer's Disease: From Pathology to Imaging. Iron and Alzheimer's Disease: From Pathology to Imaging, 16. https://doi.org/10.3389/fnhum.2022.838692

[20]
University of Texas at Austin. (2023, April 19). Newfound link between Alzheimer's and iron could lead to new medical interventions. ScienceDaily. Retrieved March 16, 2024 from www.sciencedaily.com/releases/2023/04/230419142741.htm

[21]
Wennström, M., Janelidze, S., Nilsson, K. P. R., Netherlands Brain Bank, Serrano, G. E., Beach, T. G., Dage, J. L., & Hansson, O. (2022). Cellular localization of p-tau217 in brain and its association with p-tau217 plasma levels. Acta neuropathologica communications, 10(1), 3. https://doi.org/10.1186/s40478-021-01307-2

[22]
Wilson, David M. et al. (2022). Hallmarks of neurodegenerative diseases. Cell, Volume 186, Issue 4, 693 - 714

[23]

Yuting Wu et al. ,Simultaneous Fe2+/Fe3+ imaging shows Fe3+ over Fe2+ enrichment in Alzheimer’s disease mouse brain.Sci. Adv.9,eade7622(2023).DOI:10.1126/sciadv.ade7622

[24]
University of Texas at Austin. (2023, April 19). Newfound link between Alzheimer's and iron could lead to new medical interventions. ScienceDaily. Retrieved March 16, 2024 from www.sciencedaily.com/releases/2023/04/230419142741.htm