

The Effects of Neuroinflammation in Early Alzheimer's Disease on Iron Homeostasis

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Abstract

Cellular iron export through ferroportin is inhibited by hepcidin, which is increased by inflammation in the body. This protective mechanism reduces blood iron levels and prevents invading micro-organisms from gaining access to iron. In early Alzheimer's disease, there is inflammation which may activate this mechanism. Therefore, iron-related biochemical values might provide an early diagnostic tool in recognizing Alzheimer's disease before there is permanent neurocognitive damage.

Introduction

Neuroinflammation begins in early Alzheimer's disease before irreversible neurocognitive damage. It may provide early diagnostic opportunities, allowing intervention before permanent neurocognitive impairment occurs.

In inflammation, the protective mechanism to sequester iron works through hepcidin, which inhibits iron-exporter ferroportin. This sequestering also affects serum ferritin and transferrin levels, which store and transport iron. (1)

This same protective mechanism may occur in the neuroinflammation of early Alzheimer's disease, allowing the use of these biochemical markers for diagnostic purposes.

Methods

Origination of Samples and Patient Data

Blood samples and longitudinal clinical data from a Layton Aging & Alzheimer's Research Center patient cohort were used, including 57 samples from 44 Alzheimer's patients (30M, 14F; all Caucasian) and 31 samples from 21 control patients (8M, 13F; all Caucasian). Clinical data included socioeconomic status, years of school, age of death, diagnosis, and MMSE (mini-mental state exam) scores. Experimental results from blood samples were not matched to longitudinal clinical data until after collection/calculations.

In addition, serum hepcidin and ferritin values for the samples were measured by Dr. Sternberg's lab.

Experimental Data from Patient Samples

Blood samples were thawed at room temperature and then centrifuged to separate the plasma which was pipetted into 96-well plates.

Quantitative colorimetric determination of iron levels and unbound iron-binding capacity (UIBC) was done using a BioTek EL808 absorbance reader, and a ferrozine Stanbio kit with neocuproine to prevent copper interference, hydroxylamine to reduce iron to its ferrous form, and hydrochloric acid to release iron. Blood levels of samples were determined using standard calculations. (2)

The sum of serum iron and UIBC is equal to total iron-binding capacity (TIBC), an indirect measure of transferrin. Samples that had sufficient volume were tested twice with this methodology.

Analysis of Collected Information

Experimental data was integrated with the longitudinal clinical information on the corresponding patients, as well as with the data collected by Dr. Sternberg's team, and examined and analyzed using Stata.

Results

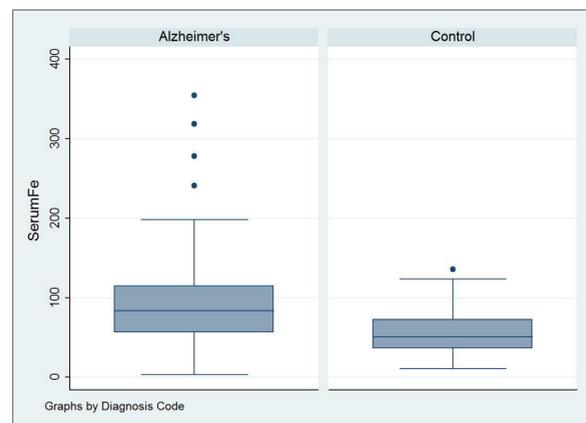


Figure 1: Serum iron levels in AD and control patients in ug/mL

Serum iron levels were found to be higher in the Alzheimer's group than in the control.

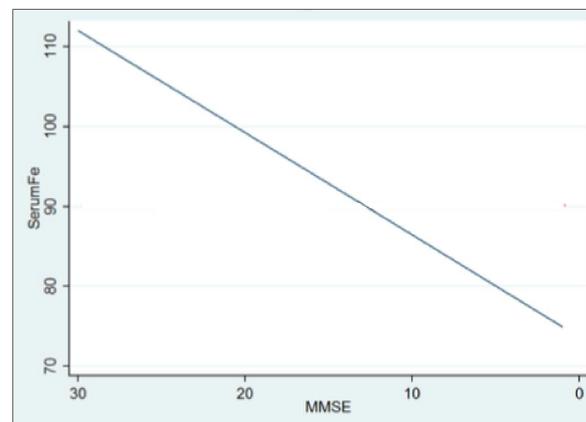


Figure 2: Serum iron levels in AD patients in ug/mL vs. MMSE score

Serum iron levels declined as MMSE score declined in the Alzheimer's group, whereas serum iron levels increased as MMSE score declined in the control group. Decreasing serum iron levels in the Alzheimer's group may be due to the action of increasing levels of serum hepcidin, which cause iron to be sequestered away from circulation as part of the inflammatory protection response.

AD group – TIBC: 344 ug/mL
Control group – TIBC: 288 ug/mL
The Alzheimer's group showed no change in TIBC (transferrin) as MMSE declines due to Alzheimer's progression

Table 1: Total Iron-binding Capacity in AD/Control groups

The Alzheimer's group was found to have higher TIBC levels which remained steady while MMSE scores declined. The control group had lower TIBC levels, but they increased as MMSE scores declined.

The Alzheimer's group also had a higher serum iron to TIBC ratio, or indirectly, transferrin saturation, than the control group (28% vs. 20.5%).

These results contrast with findings in an Australian Imaging, Biomarkers and Lifestyle Flagship Study of Aging cohort which found that their Alzheimer's group had lower transferrin saturations than controls. However, it is important to note that samples were analyzed using size exclusion chromatography-inductively coupled plasma-mass spectrometry in that study. (3)

However, the trend of decreasing TIBC levels in the Alzheimer's group as MMSE score declines may be indicative of the initiation of mechanisms to sequester iron.

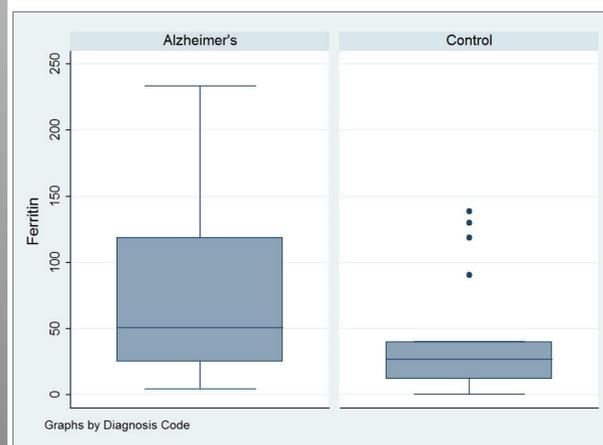


Figure 3: Ferritin levels in AD and control patients in ng/mL

Ferritin levels were markedly higher in the Alzheimer's group, compared to the control group. Ferritin stores iron and is increased during inflammation in order to sequester iron. Increased ferritin levels indicate that this mechanism may be active in Alzheimer's disease.

The serum iron:ferritin ratio was lower in the Alzheimer's group, compared to the control group, showing an excess of ferritin in the Alzheimer's group (3.17 vs. 8.03)

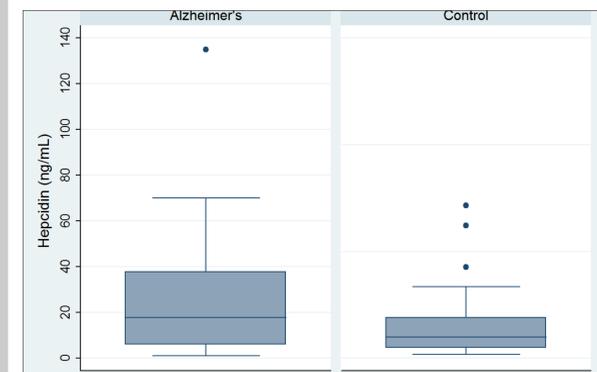


Figure 4: Hepcidin in Alzheimer's disease vs. controls in ng/mL

Hepcidin levels in the Alzheimer's group were elevated compared to controls, supporting the hypothesis that there is an activation of the hepcidin-mediated iron-sequestration mechanism in Alzheimer's disease and neuroinflammation, and that aging also involves inflammation

Hepcidin levels also increased in Alzheimer's patients as MMSE scores declined, possibly indicating that the body continues to use hepcidin as Alzheimer's progresses, and that rising hepcidin levels may be responsible for the trend of decreasing serum iron levels in the Alzheimer's group

Conclusions

There is an increase in hepcidin in Alzheimer's disease, possibly due to neuroinflammation. Likewise, there is an increase in ferritin, and steady transferrin levels in Alzheimer's, indicating that the iron-sequestering response to inflammation may be active in Alzheimer's disease.

Although serum iron is elevated in Alzheimer's patients compared to controls, the steady decrease in serum iron levels during MMSE decline in the Alzheimer's group, may likely be attributable to the increasing action of hepcidin, and other hepcidin-influenced iron-related factors.

Although the levels of these biochemical markers in Alzheimer's patients may not be clearly distinguishable from control patients in early stages, there is an increasing difference of values as Alzheimer's progresses, which may support diagnosis in the early-middle stages of neurocognitive decline.

Acknowledgements

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