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# Relationships between typical histopathological hallmarks and the ferritin in the hippocampus from patients with Alzheimer's disease

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Oxidative stress is one of the possible mechanisms of neurodegeneration. One of the elements of this mechanism are altered iron homeostasis and changes concerning of iron metabolism regulatory proteins. The primary iron storage protein in cells is ferritin, composed of heavy (H) and light (L) chains. In brain tissue neurons contain mainly ferritin H-chains, whereas glial cells are rich in L-chains. To the best of our knowledge, this is the first study that compares structure of ferritin and histopathological hallmarks in hippocampal tissue affected by the pathological process of Alzheimer's disease (AD). Our data indicate a statistically significant correlation between the concentration of L chains of ferritin, the H/L ratio and the amount of senile plaques in the subiculum, CA1 and CA4 sectors of the hippocampus ($p<0.001$, $p=0.025$, $p=0.029$). A significant correlation was also found between the concentration of L-ferritin and neuronal loss ($p=0.0026$). These findings indicate an important role of ferritin light chains in neurodegeneration, that is linked to chronic inflammation processes and the associated activation of the microglia rich of L chains.

Key words: Alzheimer's disease, hippocampus, senile plaques, neuronal loss, H and L chains of ferritin

## INTRODUCTION

Alzheimer's disease (AD) is a chronic, progressive, age-related neurodegenerative disease and the most common form of dementia in the elderly, with considerable clinical and social consequenses.

The pathological process in AD mainly affects the medial temporal lobe, that consists of hippocampal formations and associative neocortical structures. The pathological hallmarks of AD are represented by senile plaques and neurofibrillary tangles. Senile plaques, localized extracellulary in brain tissue, mainly contain amyloid-beta peptide (Aβ). Neurofibrillary tangles are composed of helical filaments. The latter are formed by the hyperphosphorylated microtubule-associated protein tau.

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The pathomechanism of neurodegeneration in AD is still not precisely known, although several hypotheses have been published. Multiple factors have been considered: genetic components, chronic inflammation, dysfunction of mitochondrial structures, apoptosis, dysfunction of cytoskeletal structures and oxidative stress. The scientific literature concerning the involvement of oxidative stress reports an altered homeostasis and an excess of iron in brain tissue.

Oxidative stress is one of the possible mechanisms of neurodegeneration. One of the exponents of this mechanism is an altered iron homeostasis. It is known from many studies, that most of the iron in the human brain is stored within the ferritin shell, that is composed of H- and L-chains. Ferritin H chains are involved in the process of incorporation of divalent iron within ferritin. Ferritin L chains are responsible for the safe storing of iron in the ferric form within the structure of iron core (Santambrogio et al. 1993). Part of the iron, which is not bound

within ferritin, is potentially toxic for neuronal cells. As a substrate of the Fenton reaction it can cause the neurodegeneration and death of cells, especially when the concentration of labile iron exceeds normal conditions (Halliwell and Gutteridge 2007). This iron is a potent source of the highly reactive hydroxyl radicals, that are generated by the Fenton reaction. Oxidative damage and the antioxidant response are well described in AD (Pohanka 2013). Some data indicate, that iron accumulation can be an important source of the oxidative damage in AD (Castellani et al. 2012). There is also evidence of an altered iron homeostasis in AD, with alterations of iron metabolism regulatory proteins – like ferritin, transferrin or hepcidin (Li et al. 2011).

In our study we aimed to analyse the relationship between typical AD histopathological changes such as neuronal loss, senile plaques and neurofibrillary tangles and the heavy and light chains of ferritin concentration in the hippocampal tissue from AD patients. To the best of our knowledge this is the first study, that compares the structure of ferritin and histopatological hallmarks in the hippocampal tissue affected by the pathological process of AD.

## MATERIALS AND METHODS

The study is based on 10 brain tissue samples from patients with Alzheimer's disease, including four anatomical regions of the hippocampus. The average age of patients was 75.6±9.5 years. All samples underwent standardised neuropathological evaluation by one neuropathologist (Mayo Clinic, Jacksonville). Average brain weight was 1050±160 grams. Four areas of the hippocampus were studied (subiculum, CA1, CA2, CA4) – according to the anatomical criteria of Duvernoy (1988). Thioflavin-S fluorescence microscopy was used (an Olympus BH2 fluorescence microscope) for quantitative measurement densities of senile plaques and neurofibrillary tangles. The following procedures and the neuroanatomical sampling model used, were based on the methods developed by Terry and others (1987) for thioflavin-S fluorescence microscopy. For structural analysis 5 μm thick, formalin-fixed and paraffin-embedded sections of hippocampus tissue were stained with haematoxylin and eosin. The slides were scanned on a ScanScope XT (Apeiro) with 20× magnification and annotated with ImageScope (version 10.2). Neurofibrillary tangle counts were calculated per 0,125 $mm^2$ surface and contained intracellular and extracellular tangles. Senile plaque counts were calculated per 3 $mm^2$ and comprised all types of plaques. Neuronal counts were estimated by dividing the number of neurons by the annotated area (neurons per $mm^2$). Four points semi-quantitative scale was used to examine the extent of neuronal loss in the pyramidal layer of the hippocampus, where 0=none, 1=mild, 2=moderate and 3=severe. Thioflavin-S fluorescence microscopy was also used to assess the Braak neurofibrillary tangle stage. The degree of pathology estimated by Braak stageing was 5.3±1.1.

Contemporaneously hippocampal tissue samples obtained from the same brains, and control hippocampal tissue samples of patients without neurodegenerative disorders, were analysed for their concentration of ferritin H and ferritin L chains by the Biochemistry Laboratory of the Brodno Voivodship Hospital in Warsaw. The control group consisted of hippocampal tissue of 20 patients without history of neurodegenerative diseases (n=20, 11 – female, 9 – men, mean age=72.6±3.0). The ELISA (enzyme-linked immuno-absorbent assay) method was applied to determine the concentration of H and L ferritins and the H/L ratio, using monoclonal antibodies specific to H (rHO2) and L (LFO3) subunits of ferritin and human H- and L-recombinant ferritins as standards (Luzzago et al. 1986, Arosio et al. 1990, Thorpe et al. 1997). Ferritin levels were calculated as ng/μg protein and as ng/mg of wet tissue.

The brain tissue samples derived from Mayo Clinic Florida Brain Bank, which operates under protocols approved by Mayo Clinic IRB. De-identified human brain samples from deceased persons are considered except from human subjects research regulations. In addition, we received permission of Bioethics Committee of the Medical University of Warsaw, for studying the ferritin's structure in brain tissues patients with AD/KB/37/2003/.

## RESULTS

The average concentration of ferritin H in the samples of AD hippocampal tissues was 19.36±4.78 ng/μg protein (367.24±80.25 ng/mg of wet tissue), while the average concentration of ferritin L – was 1.39±0.78 ng/μg protein (23.69±13.79 of wet tissue). The calculated mean H/L ratio was 19.11±11.33 (see Table I). In the control group, consisting of hippocampus tissues from patients without a history of neurodegenerative

Table I

The table shows the results of the ferritin's H and L chains concentration in hippocampal tissue from 10 patients with Alzheimer's disease, obtained using ELISA method, calculated in ng/µg protein and ng/mg wet tissue

| NPID | Ferritin H ng/µg protein | Ferritin H ng/mg wet tissue | Ferritin L ng/µg protein | Ferritin L ng/mg wet tissue | H/L ratio |
|---|---|---|---|---|---|
| AD 1 | 27.10 | 515.29 | 1.88 | 35.74 | 14.4 |
| AD 2 | 16.62 | 273.11 | 1.01 | 16.61 | 19.4 |
| AD 3 | 28.34 | 441.39 | 2.89 | 45.06 | 9.8 |
| AD 4 | 19.10 | 368.92 | 1.47 | 28.39 | 13.0 |
| AD 5 | 15.46 | 279.57 | 0.40 | 7.30 | 38.6 |
| AD 6 | 20.40 | 340.38 | 1.55 | 25.84 | 13.2 |
| AD 7 | 15.41 | 364.32 | 2.09 | 15.41 | 7.4 |
| AD 8 | 16.81 | 273.17 | 0.47 | 7.59 | 35.8 |
| AD 9 | 19.43 | 384.96 | 0.66 | 13.17 | 29.2 |
| AD 10 | 14.91 | 431.31 | 1.44 | 41.78 | 10.3 |
| Mean | 19.36±4.78 | 367.24±80.25 | 1.39±0.78 | 23.69±13.79 | 19.11±11.33 |

diseases, the concentration of H ferritin was 5.84±2.32 ng/µg protein (101.31±39.21 of wet tissue) and L ferritin – 0.55±0.42 ng/µg protein (9.42±6.98 of wet tissue). The calculated mean H/L ratio in control group was 14.48±8.06 (Table II). The quantitative data for counts of senile plaques, neurofibrillary tangles and neuronal loss in each hippocampal areas (CA1, CA2, CA4, subiculum) is presented in Table III.

## STATISTICAL ANALYSIS

To compare concentration of ferritin H- and L-chains in the hippocampal tissue of patients with Alzheimer's disease and a control group – Mann-Whitney test was used. The concentrations of ferritin H and L were statistically higher in patients with AD (Figs 1, 2). There was not statistically significant difference in H /L ratio

Table II

The table shows the results of the ferritin's H and L chains mean concentration in hippocampal tissue from 20 patients (11 – female, 9 – men) without history of neurodegenerative diseases, obtained using ELISA method, calculated in ng/µg protein and ng/mg wet tissue

| | Ferritin H ng/µg protein | Ferritin H ng/mg wet tissue | Ferritin L ng/µg protein | Ferritin L ng/mg wet tissue | H/L ratio |
|---|---|---|---|---|---|
| HIP | 5.84±2.32 | 101.31±39.21 | 0.55±0.42 | 9.42±6.98 | 14.48±8.06 |

between the groups. Calculations were performed in the program IBM SPSS 23.0.

The Spearman correlation coefficient was used to assess the relationships between the neuropathological findings and the concentration of H and L ferritin. The results are shown in Table IV. The data obtained in our study indicate a high correlation between the concentration of L chains of ferritin and the amount of senile plaques in the subiculum, CA4 and CA1 areas. The resulting correlations are plotted in the graph (Fig. 3). A significant correlation was also found between the concentration of L-ferritin and the neuronal loss. The significant correlation between neuronal loss and senile plaques was also detectable for the H/L ratio in the samples of AD hippocampal tissue.

## DISCUSSION

The importance of iron in the pathogenesis of AD is suggested by data indicating its links with oxidative stress and by its toxicity to neurons (Smith et al. 1996). The characteristic feature of brain tissue metabolism is high oxygen consumption, thus high levels of potentially toxic free oxygen radicals, as well as antioxidant defense are to be expected. In the normal homeostatic state a balance ensuring the neutralization of free radicals is maintained. However, the pathological conditions of AD damage the molecules of proteins, sugars, lipids and nucleic acids leading to neurodegeneration (Martins et al. 1986). It is also widely recognized, that free oxygen radicals generated by abnormal iron

Table III

The table shows neuropathological data: the contents of senile plaques, neurofibrillary tangles and neuronal loss in four separate anatomical regions of hippocampus – CA1, CA2, CA4, subiculum – in hippocampus tissue samples from 10 patients with Alzheimer's disease, obtained using thioflavin-S fluorescence microscopy (Olympus BH2). Neurofibrillary tangle counts are calculated per 0,125mm$^2$ and senile plaques counts are calculated per 3mm$^2$. Neuronal counts are estimated by dividing the number of neurons by the annotated area (neurons per mm$^2$). Four points semi-quantitative scale was used to examine the extent of neuronal loss in the pyramidal layer of the hippocampus, where 0=none, 1=mild, 2=moderate and 3=severe. The average age of AD patients was 75.6±9.5 years. The acerage brain weight was 1050±160 grams

| NPID | Sex | Age | Brain weight | Braak stage | Neuronal loss | Neurofibrillary tangles | | | | Senile plaques | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | CA4 | CA2 | CA1 | Sub | CA4 | CA2 | CA1 | Sub |
| AD 1 | F | 65 | 840 | V | 2 | 2 | 2,5 | 10,5 | 17,5 | 2 | 0,5 | 17,5 | 25 |
| AD 2 | F | 81 | 940 | VI | 2 | 4 | 10 | 30 | 37,5 | 7 | 2 | 6,5 | 17,5 |
| AD 3 | M | 83 | 1060 | VI | 2 | 2 | 4 | 22 | 30 | 9 | 0 | 14 | 20 |
| AD 4 | F | 92 | 1100 | VI | 2 | 2,5 | 1,5 | 10 | 28,5 | 1 | 1,5 | 7,5 | 18,5 |
| AD 5 | M | 67 | 1380 | II–III | 0 | 0,5 | 0 | 0,5 | 0 | 0,5 | 0 | 1 | 10,5 |
| AD 6 | M | 81 | 920 | VI | 2 | 2 | 2 | 11 | 27,5 | 2 | 3 | 12,5 | 32,5 |
| AD 7 | F | 73 | 1180 | V–VI | 2 | 1,5 | 2 | 10 | 10 | 4,5 | 0 | 6,5 | 35 |
| AD 8 | M | 61 | 920 | VI | 1 | 1 | 0,5 | 6,5 | 7 | 0 | 0,5 | 5 | 11 |
| AD 9 | M | 73 | 1140 | VI | 1 | 0,5 | 0 | 1,5 | 3 | 1 | 2 | 4,5 | 7 |
| AD 10 | M | 80 | 1020 | VI | 2 | 6 | 3,5 | 14 | 16,5 | 7,5 | 5,5 | 21 | 16 |

Table IV

Correlation between the H and L chains of ferritin and neuropathological findings in AD hippocampus tissue samples, using Spearman coefficient. The data obtained, indicate a statistically significant correlation between the concentration of L chains of ferritin and the amount of senile plaques in the subiculum, CA4 and CA1 areas. A significant correlation was also found between the concentration of L-ferritin and the neuronal loss

| | Neuronal loss | Neurofibrillary tangles | | | | Senile plaques | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CA4 | CA2 | CA1 | Subiculum | CA4 | CA2 | CA1 | Subiculum |
| Ferritin H | r=0.16 | r=−0.11 | r=0.09 | r=0.16 | r=0.37 | r=0.031 | r=−0.11 | r=0.24 | r=0.24 |
| | p=0.66 | p=0.76 | p=0.78 | p=0.63 | p=0.28 | p=0.92 | p=0.73 | p=0.49 | p=0.49 |
| Ferritin L | r=0.81 | r=0.36 | r=0.58 | r=0.55 | r=0.56 | r=0.68 | r=−0.20 | r=0.68 | r=0.87 |
| | p=0.0026 | p=0.29 | p=0.074 | p=0.089 | p=0.081 | p=0.029 | p=0.56 | p=0.025 | p<0.001 |
| H/L ratio | r=−0.81 | r=−0.51 | r=−0.56 | r=−0.54 | r=−0.48 | r=−0.74 | r=0.031 | r=−0.66 | r=−0.72 |
| | p=0.0026 | p=0.12 | p=0.089 | p=0.098 | p=0.15 | p=0.011 | p=0.92 | p=0.033 | p=0.016 |

metabolism play an important role in the pathogenesis of AD. Data on iron accumulation in AD brain regions that lead to specific damaged, including the hippocampal formations, are also well known (Smith et al. 1996). Some data at the microscopic level has shown an increased accumulation of iron in the senile plaques connected with Alzheimer's disease (Exley et al. 2012). Several pieces of evidence report that the production of β-amyloid increases in oxidative stress conditions (Schilling and Eder 2011). It has also been suggested, that β-amyloid itself has the ability to stimulate generation of free oxygen radicals. Molecules of β-amyloid contain histidine residues, that have iron binding properities and these iron atoms generate free oxygen radicals via the Fenton reaction (Zheng et al. 2006). It is suggested, that β-amyloid aggregation and forming senile plaques are essential in the processes of neurotoxicity.

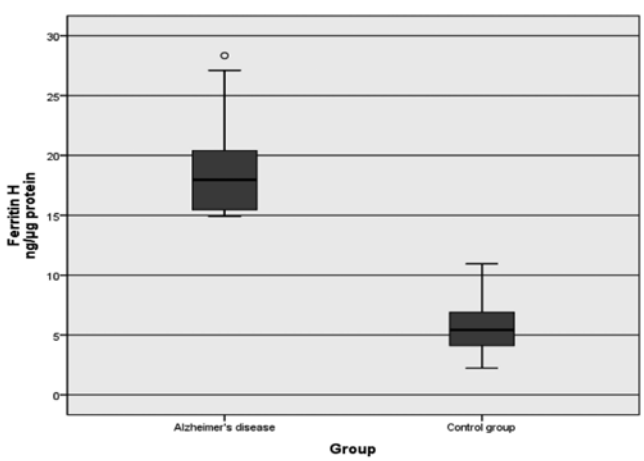


Fig. 1. The concentration of ferritin H (ng/µg protein) was statistically higher in the hippocampal tissues of patients with AD, compare to hippocampal tissues of patients without history of neurodegenerative diseases.

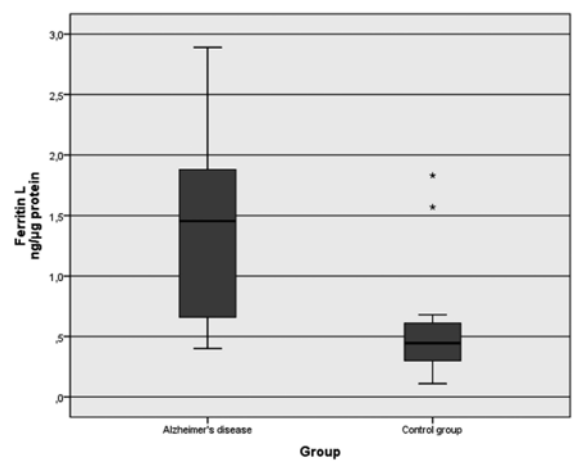


Fig. 2. The concentration of ferritin L (ng/µg protein) was statistically higher in the hippocampal tissues of patients with AD, compare to hippocampal tissues of patients without history of neurodegenerative diseases.

On the other hand the mechanism responsible for neurodegeneration is a chronic inflammation process. Senile plaques and the altered metabolism of iron are considered for stimulants of activation of microglia (Cullen 1997), the reactive astrocytes, as well as the macrophages activation, which are responsible for production of inflammatory cytokines such as Il-6, Il-1, Il-8 (Rubio-Perez and Morillas-Ruiz 2012). The process of chronic inflammation stimulates the macrophages to generate free radicals (McGeer and McGeer 1998). Furthermore the activated microglia is responsible for the release of iron deposited in ferritin which thereby exacerbates the inflammatory process in brain tissue (Vlad et al. 2008). Several pieces of evidence illustrate increased levels of Il-6, Il-1, TNFα in the brain tissue and cerebrospinal fluid of patients with AD (Rubio-Perez and Morillas-Ruiz 2012). Some epidemiological studies indicate, that the chronic use of nonsteroidal anti-inflammatory drugs, by inhibiting the enzymatic activity of the inflammatory cyclooxygenases COX-1 and COX-2, reduce the risk of developing AD in a healthy aging population (Rubio-Perez and Morillas-Ruiz 2012).

At the same time ferritin studies have shown that neurons contain mainly ferritin H-chains, whereas glial cells are rich in L-chains (Connor et al. 1995). These observations are consistent with data obtained in our study, which show that the increase of senile plaques counts, correlates with the concentration of L ferritin chains.

In our previous studies, using ELISA tests, we demonstrated a threefold increase in the H and L chain concentrations in AD as compared to the control hippocampal tissue of patients without neurodegenerative disorders. This increase of both ferritin chains was not, however, accompanied by a parallel increase of the total iron concentration which only rose by 50% compared to the control (Gałązka-Friedman et al. 2011). In our study of tissue samples hippocampus of patients with AD and patients without a history of neurodegen-

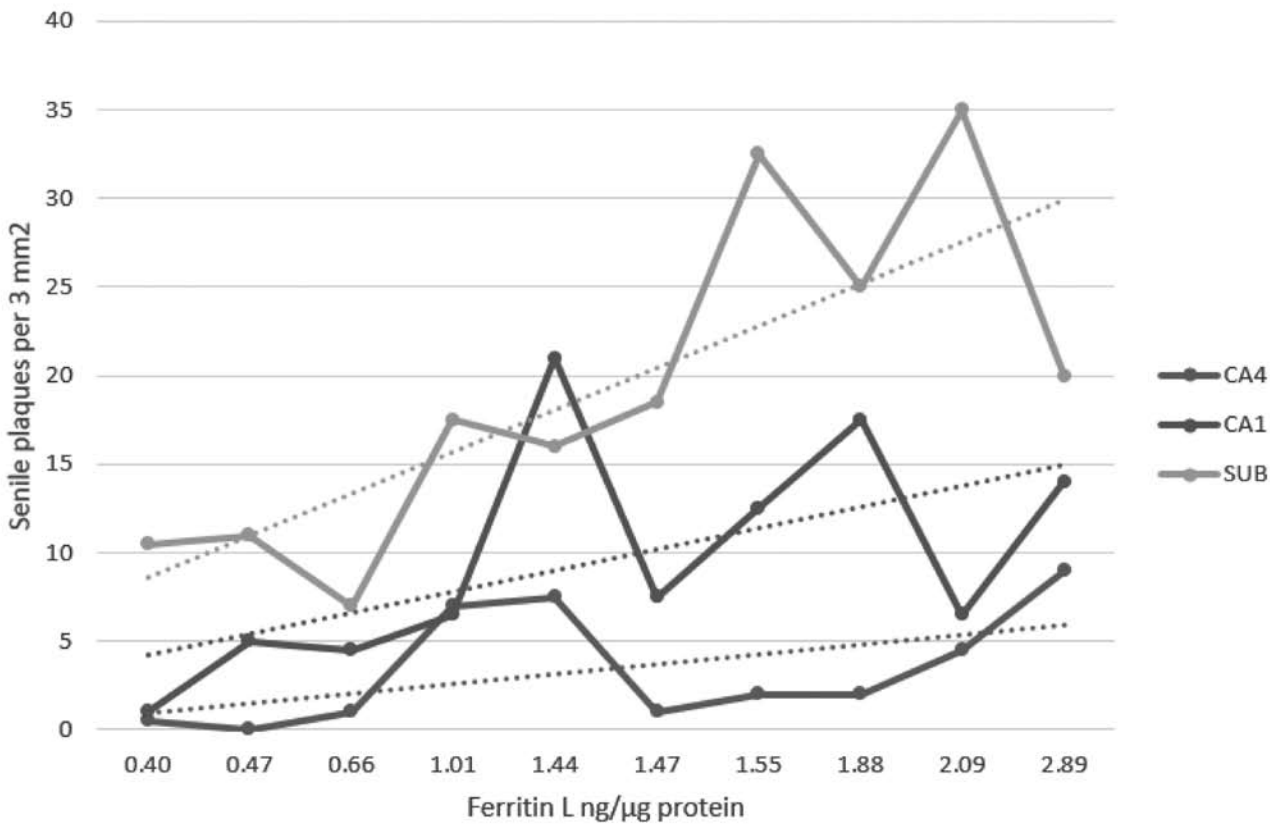


Fig. 3. The graph shows the relationships between the concentration of L chains of ferritin (ng/µg protein) and the amount of senile plaques (per 3 $mm^2$) in the separate anatomical regions of AD hippocampus: subiculum, CA4 and CA1 areas. Marked trend lines indicate a correlation between increase in the number of senile plaques with increasing concentrations of L chains of ferritin.

erative diseases using the ELISA method, we also obtained a statistically significant higher levels of ferritin in patients with AD. H/L ratio in a group of patient with AD was not statistically significant, due to the increased concentrations of both H chains and L ferritin.

The data obtained in this study indicates a high correlation particularly between the concentration of ferritin L chains, the H/L ratio and the amount of senile plaques. It seems to indicate, there is an association between high concentrations of ferritin L chains, ongoing processes of chronic inflammation, with the associated activation of the microglia reach in L chains and an increased number of senile plaques and neuronal loss in the hippocampal tissue of AD patients.

Additionally, it is interesting that the results of this study differ from the results of ferritin structure studies, obtained by us in previous studies concerning the parkinsonian substantia nigra (SN). It was found there, that a decrease of L ferritin was not associated with a change in the concentration of iron when compared to a control (Koziorowski et al. 2007). A decrease of the concentration of L ferritin in the substantia nigra in Parkinson's disease is considered a possible cause of neurodegeration. This finding was paralleled by an increase of low molecular weight iron, which could represent labile iron, in parkinsonian SN compared to control (Wypijewska et al. 2010). There is a difference in the way, that the pathological process of the two diseases (PD and AD) affects the nervous structures. These differences suggest distinic mechanisms of neurodegeneration in Alzheimer's disease and Parkinson's disease, and may suggest an important role of chronic inflammation in AD.

Convincing evidence of the involvement of ferritin in neurodegeneration is also the mechanism of neuronal degeneration in neuroferritinopathy – a rare, monogenic, autosomal dominant progressive movement disorder, that is caused by mutations in the gene encoding the L chain of ferritin (Curtis et al. 2001).

## CONCLUSIONS

The results of this study provide evidence for a relationship between the severity of neurodegeneration in hippocampal tissue affected by Alzheimer's disease pathology and changed structure of ferritin – the main iron storage protein. We found a statistically significant correlation between the concentration of light chains of ferritin and counts of senile plaques and neuronal loss. The latter are typical hallmarks of Alzheimer's disease and are found mainly in the hippocampal tissue. Enhanced concentration of light chains of ferritin may be associated with an increase in the number of microglia cells – rich in L chains of ferritin, resulting in an ongoing inflammatory process. Evidence of the important role of L-chains in neurodegeneration is also found in the case of neuroferritinopathy, caused by mutations in the gene encoding the L chain of ferritin, that belongs to the typical representatives of neurodegenerative diseases, extending clinically in the form of motor and cognitive symptoms. The differences in the structure of ferritin identified in the nervous structures affected in PD and AD cases suggest, there are differences between mechanisms of neurodegeneration in these two diseases and point to the possibility of important role of chronic inflammation in AD. An important role of iron metabolism and proteins regulating its metabolism in brain tissue occupied in pathological process in Alzheimer's disease merits further research, which will help determine its participation in the process of neurodegeneration and bring us closer to a more detailed knowledge of the pathogenesis of Alzheimer's disease.

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## REFERENCES

Arosio P, Cozzi A, Levi S, Luzzago A, Ruggeri G, Iacobello C, Santambrogio P, Albertini A (1990) A mutantional analysis of the epitops of recombinant human H-ferritin, Biochim. Biophys Acta 1039: 197–203.

Castellani RJ, Moreira PI, Perry G, Zhu X (2012) The role of iron as a mediator of oxidative stress in Alzheimer disease. Biofactors 38: 133–138.

Connor JR, Snyder BS, Arosio P, Loeffler DA, LeWitt P (1995) A quantitative analysis of isoferritins in select regions of aged, parkinsonian, and Alzheimer's diseased brains. J Neurochem 65: 717–724.

Cullen KM (1997) Perivascular astrocytes within Alzheimer's disease plaques. Neuroreport 8: 1961–1966.

Curtis AR, Fey C, Morris CM, Bindoff LA, Ince PG, Chinnery PF, Coulthard A, Jackson MJ, Jackson AP, McHale DP, Hay D, Barker WA, Markham AF, Bates D, Curtis A, Burn J (2001) Mutation in the gene encoding ferritin light polypeptide causes dominant adult-onset basal ganglia disease. Nat Genet 28: 350–354.

Duvernoy HM (1988) The human hippocampus: an atlas of applied anatomy (1th ed.) J.F. Bergmann-Verlag, Munich, Germany.

Exley C, House E, Polwart A, Esiri MM (2012) Brain burdens of aluminum, iron, and copper and their relationships with amyloid-β pathology in 60 human brains. J Alzheimers Dis 31: 725–730.

Gałązka-Friedman J, Bauminger ER, Szlachta K, Koziorowski D, Tomasiuk R, Jaklewicz A, Wszołek ZK, Dickson D, Kaplińska K, Friedman A (2011) Iron in Alzheimer's and Control Hippocampi – Mösssbauer, Atomic Absorption and ELISA Studies. Acta Phys Pol A 119: 81–83.

Halliwell B, Gutteridge JMC (2007) Free Radicals in Biology and Medicine (4th ed.) Oxford University Press, Oxford, UK.

Koziorowski D, Friedman A, Arosio P, Santambrogio P, Dziewulska D (2007) ELISA reveals a difference in the structure of substantia nigra ferritin in Parkinson's disease and incidental Lewy body compared to control. Parkinsonism Relat Disord 13: 214–218.

Li L, Holscher C, Chen BB, Zhang ZF, Liu YZ (2011) Hepcidin treatment modulates the expression of divalent metal transporter-1, ceruloplasmin, and ferroportin-1 in the rat cerebral cortex and hippocampus. Biol Trace Elem Res 143: 1581–1593.

Luzzago A, Arosio P, Iacobello, C, Ruggeri G, Capucci L, Brocchi E, De Simone F, Gamba D, Gabri E, Levi S, Albertini A (1986) Immunochemical characterization of human liver and heart ferritins with monoclonal antibodies. Biochim Biophys Acta 872: 61–71.

Martins RN, Harper CG, Stokes GB, Masters CL (1986) Increased cerebral glucose-6-phosphate dehydrogenase activity in Alzheimer's disease may reflect oxidative stress. J Neurochem 46: 1042–1045.

McGeer EG, McGeer PL (1998) The importance of inflammatory mechanisms in Alzheimer disease. Exp Gerontol 33: 371–378.

Pohanka M (2013) Alzheimer´s disease and oxidative stress: a review. Curr Med Chem 21: 356–364.

Rubio-Perez JM, Morillas-Ruiz JM (2012) A review: inflammatory process in Alzheimer's disease, role of cytokines. ScientificWorldJournal 2012: 756357.

Santambrogio P, Levi S, Cozzi A, Rovida E, Albertini A, Arosio P (1993) Production and characterization of recombinant heteropolymers of human ferritin H and L chains. J Biol Chem 268: 12744–12748.

Schilling T, Eder C (2011) Amyloid-β-induced reactive oxygen species production and priming are differentially regulated by ion channels in microglia. J Cell Physiol 226: 3295–3302.

Smith MA, Perry G, Richey PL, Sayre LM, Anderson VE, Beal MF, Kowall N (1996) Oxidative damage in Alzheimer's. Nature 382: 120–121.

Terry RD, Hansen LA, DeTeresa R, Davies P, Tobias H, Katzman R (1987) Senile dementia of the Alzheimer type without neocortical neurofibrillary tangles. J Neuropathol Exp Neurol 46: 262–268.

Thorpe SJ, Walker D, Arosio P, Heath A, Cook J, Worwood M (1997) International collaborative study to evaluate a recombinant L ferritin preparation as an International Standard. Clin Chem 43: 1582–1587.

Wypijewska A, Galazka-Friedman J, Bauminger ER, Wszolek ZK, Schweitzer KJ, Dickson DW, Jaklewicz A, Elbaum D, Friedman A (2010) Iron and reactive oxygen species activity in parkinsonian substantia nigra. Parkinsonism Relat Disord 16: 329–333.

Vlad SC, Miller DR, Kowall NW, Felson DT (2008) Protective effects of NSAIDs on the development of Alzheimer disease. Neurology 70: 1672–1677.

Zheng L, Rober K, Jerhammar F, Marcusson J, Terman A (2006) Oxidative stress induces intralysosomal accumulation of Alzheimer amyloid beta-protein in cultured neuroblastoma cells. Ann N Y Acad Sci 1067: 248–251.