

Brain iron: comparison of postmortem SWI with chemical tissue analysis

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Introduction:

Brain iron is believed to play a role in pathophysiology of neurodegenerative processes. Increased iron levels in certain regions of the brain have been reported in various diseases including Alzheimer's and Parkinson's disease or multiple sclerosis as well as TBI or ageing [1, 2]. Numerous publications have investigated whether non-invasive assessment of brain iron is possible by using MRI [3]. However, validation with a gold standard from chemical analysis of brain tissue is essential. Most studies still refer to the work of Hallgren and Sourander [4] from 1958. Since then chemical analytical methods went through an enormous process. To provide an up to date basis for comparison with quantitative MR parameters iron concentration was determined in selected brain regions using inductively coupled plasma mass spectrometry. First results are reported and compared to susceptibility weighted imaging (SWI) [5].

Methods:

Five deceased male subjects (mean age 57.2 y, range 38-81) without known neurological deficits or disease were measured within 72 hours after death on a 3T Tim Trio (Siemens Healthcare, Erlangen, Germany) using a spoiled FLASH sequence for SWI contrast generation [5] (TE/TR=20/30ms, matrix=512x512, in-plane resolution=0.5x0.5mm², slice thickness 2 mm, NEX=2). Autopsy was performed within 14 hours after the scan, whole brains were removed and fixed in 4% neutral buffered formalin for at least three weeks. Tissue samples (Fig. 1) from globus pallidus (GP), putamen (Put), caudate nucleus (CN), thalamus (Thal), corpus callosum (CC), pons, red nucleus (RN) and frontal, temporal and occipital white matter (FWM, TWM, OWM) were dissected from 1 cm thick transversal slices. For all procedures a ceramic knife was used to avoid iron contamination and specimens were weighted before and after freeze-drying. Freeze-dried tissue samples were mineralized with nitric acid in a microwave heated autoclave (UltraCLAVEIII, EMLS, Leutkirch, Germany). Iron concentration was determined with an inductively coupled plasma mass spectrometer (ICPMS) (Agilent 7500ce, Agilent Technologies, Waldbronn, Germany) at m/z 56 in He-mode. The accuracy of the results was checked with the NIST bovine muscle (RM 8414). The study was approved by the local ethics' committee and informed consent of near relatives was obtained.

Results:

Fig. 2 shows the mean iron concentration of the different brain regions separately for both hemispheres. Each column represents the mean of all five cases, whiskers stand for the range between minimal and maximal value. Highest concentrations were found in the basal ganglia: globus pallidus (mean right/left=191/219 mg/kgww (wet weight), range 158-243 mg/kgww), putamen (mean right/left=142/151 mg/kgww, 103-178 mg/kgww) and caudate nucleus (mean right/left=82/91 mg/kgww, 58-98 mg/kgww). In the red nucleus (RN) one single case had exceptional high values (156 mg/kgww, both sides) which are shown separately by asterisks, while all other cases had relatively low values (mean right/left=24/26 mg/kgww, 14-34 mg/kgww). A tendency to slightly higher values in the left hemisphere compared to the right side is observed in all examined regions except for thalamus and pons. Fig. 3 shows the example of a postmortem SWI in situ of a 58 year old male subject, related iron concentrations are listed in Tbl. 1. Visual comparison of the SWI image contrast with the measured iron concentrations of certain regions demonstrates that regions with high iron content correlate well with hypointense regions.

Discussion and Conclusion:

Using postmortem brain tissue, results of MR imaging methods can be validated by chemical analysis within the same individual. The iron concentrations obtained by ICPMS are in good agreement with the values published by Hallgren and Sourander [4]. However, red nucleus values are much lower compared to Hallgren's (195 mg/kgww) with the exception of one case. As this is the only region where such a discrepancy was observed, a systematic error seems rather unlikely and the reason is not clear so far. The slight tendency that in most brain regions mean iron concentration in the left hemisphere is greater than in the right hemisphere is interesting. However, inter-individual differences in vascularisation, hem content inside vessels, peripher vascular congestion and the small number of subjects investigated need to be taken into account. The visual agreement of measured iron concentrations with SWI image contrast in specific brain regions supports the suggestion of previous studies that brain iron could be evaluated by SWI [3]. Further work will focus on correlating SWI contrast and phase information with iron concentration quantitatively.

References:

[1] Haacke EM et al, Magn Reson Imaging 2005, 23:1; [2] Stankiewicz J et al, Neurotherapeutics 2007, 4:371; [3] Zhu WZ et al, Radiology 2009, 253:497 [4] Hallgren B, Sourander P, J Neurochem 1958, 3:41; [5] Reichenbach JR et al, J Comput Assist Tomogr 2000, 24:949

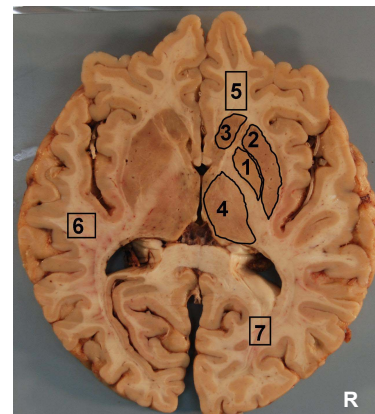


Fig. 1 Fixed brain slice with analyzed regions: 1 globus pallidus, 2 putamen, 3 caudate nucleus, 4 thalamus, 5 frontal WM, 6 temporal WM, 7 occipital WM (not shown: corpus callosum, pons and red nucleus)

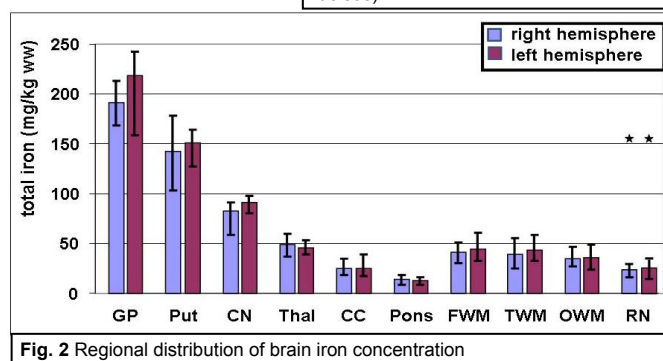


Fig. 2 Regional distribution of brain iron concentration

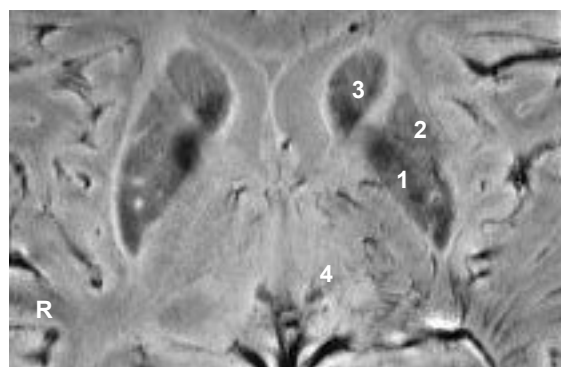


Fig. 3 Detail of postmortem SWI in situ of a 58y old male subject

Tbl. 1 Brain iron concentrations of 58y old male [mg/kgww]

| | right | left |
|-------------------|-------|-------|
| 1 globus pallidus | 211,5 | 242,5 |
| 2 putamen | 135,4 | 155,3 |
| 3 caudate nucleus | 91,0 | 93,4 |
| 4 thalamus | 42,6 | 41,2 |