Correlation between paramagnetic ions and quantitative susceptibility values of postmortem brain study

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Target Audience: People interested in the relationship between quantitative susceptibility values and paramagnetic ions deposit in the brain.

Introduction: Histochemical and biochemical studies of neurodegenerative diseases have found elevated concentrations of metals in the postmortem brain, specially, Fe and the paramagnetic ion Fe^{+3} on the substantia nigra in Parkinson's disease[1,2]. But these reports did not show the cause of the highest metal concentration and even less about the stage of disease that happening the accumulation. The metal quantification in vivo allows to understand the relationship between metal deposition and the disease progression. Quantitative susceptibility mapping (QSM) have been suggested as the most sensible technique in vivo to quantify metals concentration in the brain[3,4]. However, QSM studies evaluated the sensibility only of total iron[3,5], but the tissue have others metals and in different paramagnetic states[1]. We highlight the paramagnetic ions because have the most influence on susceptibility value. Electron Paramagnetic Resonance (EPR) is an excellent technique to quantify paramagnetic ion concentrations and also give information about the paramagnetic center's electronic structure. Our goal is to study QSM maps of postmortem brain tissues and its correlation with magnetic species concentration.

Methods: Magnetic resonance imaging were performed in two human postmortem brain in situ (one women of 58 years and one men of 64 years) using a 3T scanner. The postmortem interval was shorter than 15 hours. The subjects did not have history of neurological disorders. The local ethics committee approved the study and informed consent was obtained from each individual's next of kin. The brains were removed on the autopsy service and fixed in paraformol solution (4%) by more of 150 days. For QSM a gradient multi-echo sequence (spatial resolution: 0.479x0.479x2.0 mm^3) was used with 4 equally spaced echoes (TE=7.7,19.7,31.7, 43.7 msec) . We obtained the susceptibility maps using the MEDI algorithm[6]. Several ROIs were drawn in the basal ganglia for left and right side: substantia nigra (SN), red nucleus (RN), globus pallidus (GP), putamen (PUT), caudate nucleus (CN), thalamus (THA) and white matter (WM). For each region, a tissue sample was placed in a glass tube (inner diameter of 3mm) within another tube with liquid nitrogen. Electron Paramagnetic Resonance (EPR) spectra was recorded in an X-Band (Jeol JES-FA-200) spectrometer with a central magnetic field of 250mT, scanning field of 150mT, scan time of 4 minutes, modulation amplitude of 1mT, gain of 50 and microwave power of 2mW. Simulations of EPR spectrum were performed using the EasySpin® toolbox [7] to quantify the paramagnetic ions in the experimental spectrum (g-value and respective amplitude peak-to-peak normalized by mass). **Results:** Figure 1 shows T2* weighted images and susceptibility maps for a postmortem brain in situ. All brain regions studied had four main resonances on the EPR spectrum with different amplitudes between them (Basal ganglia, Figure 2). Figure 3 shows the correlation between paramagnetic ions with susceptibility values for the two postmortem cases. **Discussion and Conclusion:** The EPR spectrum showed g=5.8 of high-spin Fe^{+3} that is present in haem proteins as methemoglobin[8,10-11]; g= 4.1 of high-spin Fe^{+3} that is present in no-haem proteins as transferrin and ferritin[8-11]; g∥=2.19 and g□=2.003 are from Cu^{+2} that is present in ceruloplasmin[11]. We observed linear correlation between susceptibility value and amplitude peak-to-peak from EPR spectrum normalized by mass only for Fe^{+3}(g=4.1) (R^2>0.64). These results confirm the sensibility of QSM to measure iron ion. More subjects will be evaluated to confirm the results. We also will measure the total metals concentration of respective brain regions by ICP-MS spectromety to compare with EPR results. Therefore, QSM showed sensitive and specific only for the paramagnetic ion Fe^{+3} present in no-haem proteins as transferrin and ferritin.

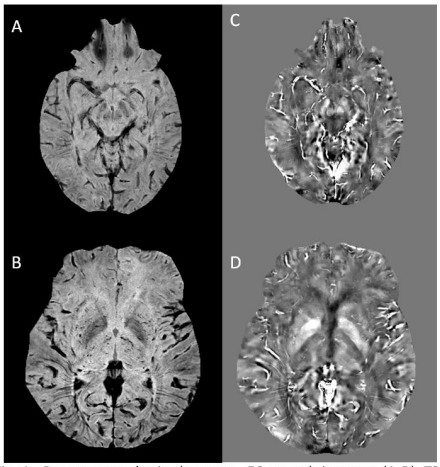

Fig 1. Postmortem brain (woman, 58 years) images: (A,B) T2* weighted images (TE=22ms); (C,D) QSM (scale -0.1 to 0.1 ppm).

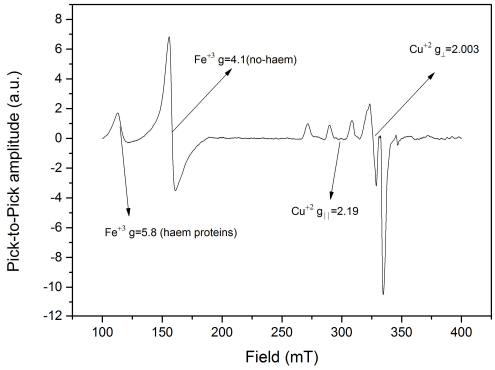

Fig. 2: Experimental EPR spectrum for a region from basal ganglia (SN).

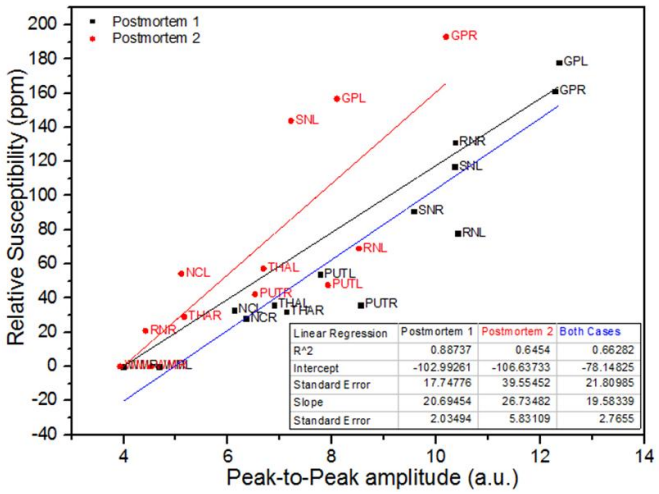

Fig. 3: Linear correlation between susceptibility value and amplitude peak-to-peak from EPR spectrum normalized by mass only for Fe^{+3}(g=4.1).

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