Validation of susceptibility mapping for quantification of iron in subcortical grey matter in multiple sclerosis

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PURPOSE: Iron accumulation in human brain has been reported in various neurological diseases including multiple sclerosis (MS), where there is increasing interest in subcortical grey matter (GM). Validating MRI measurements of human brain iron requires postmortem study. Using healthy subjects, Langkammer et al. found high correlations for both R2* mapping (1) and quantitative susceptibility mapping (QSM) in postmortem subcortical GM compared to chemical iron analysis (2). However, few studies in patients have been performed using both MRI and histology. Recently, we examined subcortical GM in postmortem MS subjects, comparing Perls' ferric iron stain to in situ MRI measurements of R2 and R2* transverse relaxometry and phase imaging, finding the highest correlations with R2* mapping (3). Here, we extend this work to QSM, a promising alternative method for brain iron mapping, and compare QSM performed in situ in postmortem MS subjects to Perls' iron stain.

METHODS: *MRI*: Three-dimensional multiple gradient-echo acquisitions were collected at 4.7T (Varian, Palo Alto, CA) from two postmortem MS patients <28hrs after death (male, age: 60 and 63 yrs) and four healthy male subjects (age: 47±2 yrs). Acquisition parameters: FOV = 256×128-160×160 mm; spatial resolution 1×0.8-1×2 mm; 80 axial slices; TR = 44 ms; 10 echoes with echo spacing 4.1 ms; TE_1 = 2.9-3.2 ms; flip angle = 10°. In addition, standard two-dimensional fast spin echo (FSE) was performed for assessing brain anatomy. A birdcage head coil was used for transmission and a tight-fitting 4-channel array coil for signal reception.

Reconstruction: Susceptibility maps were reconstructed from the multiple gradient echo datasets using the phase images from the first 5 echoes. Following receiver coil phase-offset removal and combination, phase images were unwrapped and fitted with echo times to produce a single field map. Background field removal was then performed using a new method based on the SHARP technique ("Sophisticated Harmonic Artifact Reduction for Phase data") (4). The new method known as RESHARP ("Regularization Enabled SHARP") uses Tikhonov regularization with the method of Lagrange multiplier to improve the background artifact removal, particularly at the brain boundary. After this, dipole inversion for QSM was performed using the total variation (L1 norm of gradients) regularization approach (5). For additional comparison to QSM, R2* maps were also reconstructed as previously described (6), using all 10 echoes and a linear field gradient correction.

Iron stains: The postmortem brains were extracted and fixed in 18% formalin then sectioned in 8 mm slices (1 in axial, 1 in coronal orientation) and photographed before iron staining. Slices containing subcortical GM were stained with the Perls' iron reaction. The sections were placed in a plastic container with 1 liter of 2% hydrochloric acid combined with 1 liter of 2% potassium ferrocyanide for 30 minutes. After washing for 2 minutes with water, the sections were photographed again.

Measurements: For the postmortem brains, photographs were converted to grayscale and aligned between stained and unstained brain slices. ROIs were drawn around iron-rich subcortical GM structures and the intensities from stained and unstained photographs were subtracted and divided by the difference between the background and the normal appearing white matter, generating a relative optical density, where a higher optical density corresponds to more iron staining for a given subject. MRI images (FSE, R2*, QSM) were then matched properly to photographs, and ROIs were drawn on the FSE images and transferred to R2* and susceptibility maps. Six subcortical GM structures were evaluated: globus pallidus (GP), putamen (PU), caudate (CN), thalamus (TH), red nucleus (RN) and substantia nigra (SN), (RN not present on photograph of subject 2). Each structure was measured on left and right sides and on multiple slices whenever possible.

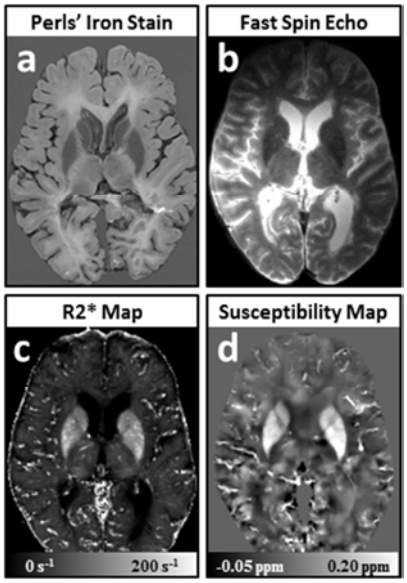

Figure 1: In situ postmortem (60 year old male)

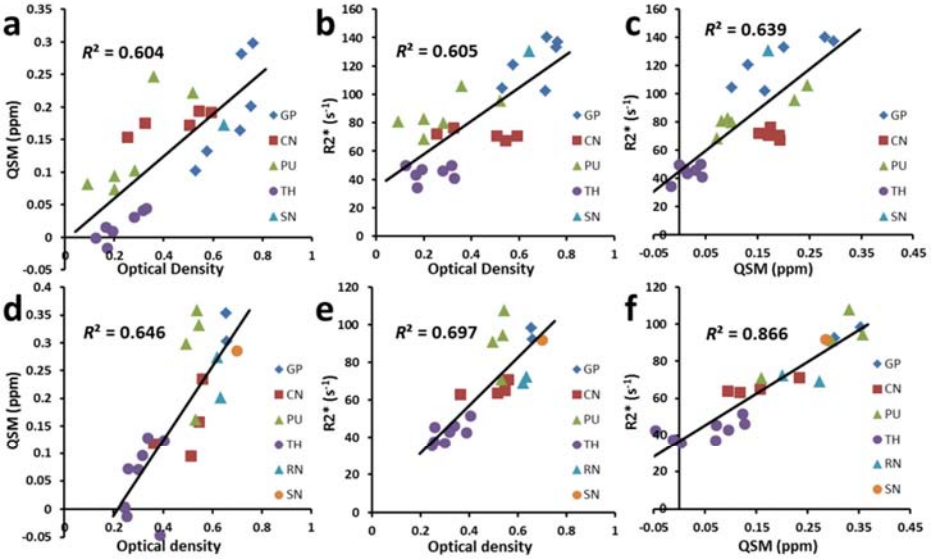

Figure 2: Linear regressions: top row subject 1, bottom row subject 2; (a,d) QSM to optical density from Perls' iron stain; (b,e) R2* mapping to optical density; (c,f) QSM to R2* mapping.

RESULTS: Example postmortem images are shown in Fig 1. Subcortical GM is well depicted with all methods: hypointense in FSE (Fig. 1b) and hyperintense in R2* and QSM (Fig. 1c,d) with good correspondence to the Perls' iron photograph (Fig. 1a). The R2* and QSM maps show very consistent patterns in terms of strong susceptibility sources from non-heme iron in deep GM and heme iron in deoxygenated vessels. Both QSM and R2* mapping have significant linear correlations ($R^2 > 0.6$) to Perls' iron stain (optical density) (Fig. 2). The R2* and susceptibility maps also exhibit significant linear correlations with each other (Fig. 2c,f). Example in vivo images are shown in Fig 3 where the pulvinar thalamus in particular shows high susceptibility contrast in QSM. R2* and QSM have a high linear correlation to each other with R^2 = 0.827, including measurements from four individuals.

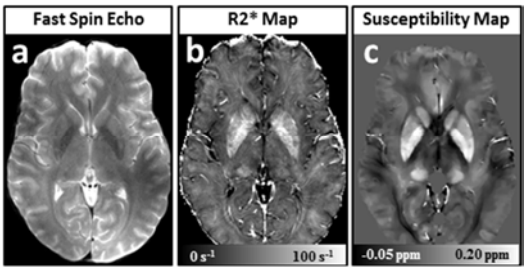

Figure 3: In vivo example of 45 yr healthy male subject.

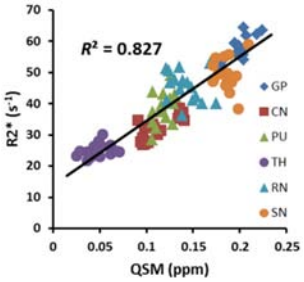

Figure 4: Linear regression of QSM to R2* mapping in vivo.

CONCLUSION: In situ postmortem imaging is free of confounding effects from formalin fixation and retains air-tissue interfaces, but is limited by full blood deoxygenation. Nevertheless R2* mapping and QSM had significant linear correlations to ferric iron staining in both postmortem MS subjects. These correlations suggest both MRI methods could be used to indicate iron status of subcortical GM in MS.

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