Quantitative properties (water content, relaxometry, MT) of the post mortem brain: a baseline for normal tissue

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Target Audience Scientists using MRI of fixed tissue.

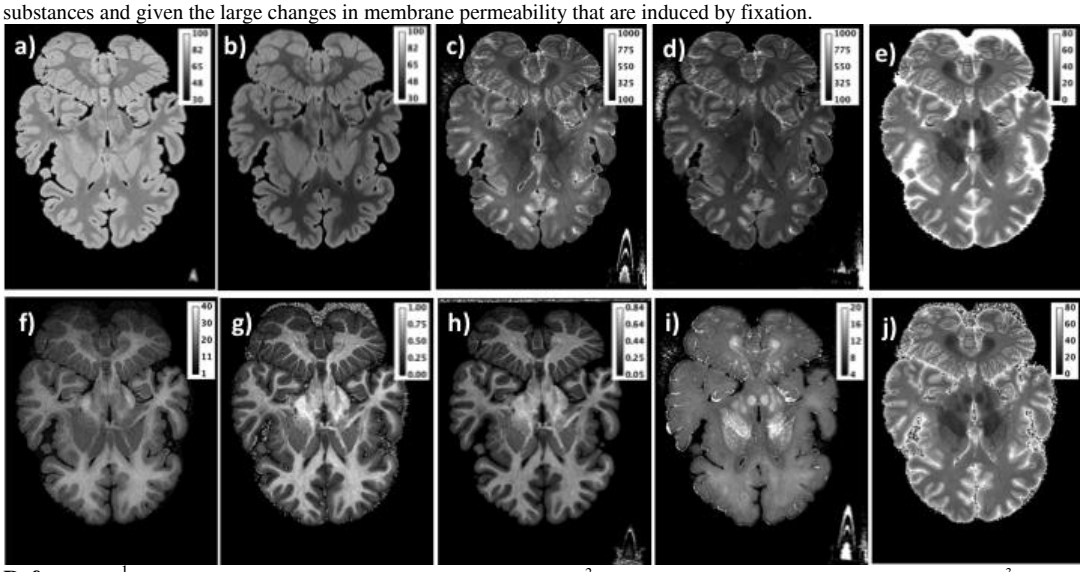
Purpose: MRI of fixed tissue is an excellent way to study pathological changes caused by different diseases with great anatomical detail. It is, however, known that properties of tissue change with fixation. The aim of this study was to investigate post mortem formalin-fixed brains quantitatively in order to provide a baseline for MR parameters characteristic of healthy brain tissue. For this, it was necessary to determine the variability of several quantitative MRI (qMRI) parameters in fixed brain tissue obtained from donors unaffected by neurological conditions and to investigate the existence of quantitative parameters which vary little between specimens. Among other parameters, quantitative water content determined non-invasively with MRI is reported on whole human post mortem brains for the first time to our knowledge. Correlations between different parameters were investigated and compared to the same quantities measured *in vivo*.

Methods: Seven whole, fixed humans brains (3 male, 4 female, aged between 47 and 79 years, mean age 67 years) were investigated. The brains were obtained in accordance with the requirements of the local ethics committees from the brain donor programmes of the Universities of Düsseldorf and Aachen. All donors were free from neurological disorders. The brains were fixed by immersion in 10% neutrally buffered formalin solution (4% formaldehyde concentration) with a mean delay between time of death and beginning of fixation of 21 hrs (between 15.5 and 26 hrs). The mean fixation time was 6 ½ months (from 2 ½ to 13 months).

For *in vivo* scans, informed consent was obtained from ten 26-year old male volunteers, in accordance with the requirements of the local ethics committee. All measurements were performed using a whole-body 3T scanner (Siemens Tim Trio), equipped with a gradient coil with maximum field strengths of 40mT/m on each axis. A body coil was used for RF transmit and a 12-element, phased-array head coil was used for signal detection. The scanning protocol for quantitative imaging consisted of 2 sets of 2-point 3D mapping protocols, one with and one without magnetisation transfer preparation, and one AFI scan[1] for B1+ mapping. The well-known signal equation for spoiled 3D gradient echo was employed for T1 and M0 parameter fitting (see e.g. [2]) and a mono-exponential fit of signal intensity as a function of echo time delivered T2* values. This was done independently for the MT-prepared scans. The qMRI parameters determined include relaxation rates T1 and T2*, MT ratio (MTR) and T1 and T2* after MT. From these we can further derive semiquantitative MT parameters such as the exchange rate (k_{trans}=MTR/$T1^{sat}$) and bound pool fraction (f_{bound}=MTR/T1) [3]. The scan parameters were optimised separately for post mortem and for *in vivo* parameter mapping. For post mortem scanning, the parameters included: TR=52ms, α=17 and 75deg, BW = 140Hz/px, TE_1 = 4.31ms, ΔTE = 8.38ms, 6 or 4 (MT preparation) echoes, 2 averages, 4 separate repetitions. Depending on the available measurement time, the resolution was 0.35x0.35x0.5 mm3 (2 brains), 0.35x0.35x0.6mm3 (3 brains) or 0.52x0.52x0.6 mm3 (2 brains). All separate scans were coregistered off-line and scans with same parameter combinations were averaged. The parameters of the *in vivo* 3D GRE acquisitions included: TR=50ms, α= 7 and 40 deg, 12 echoes, TE_1 = 2.33ms, ΔTE = 2.89ms, BW=300Hz/px, voxel size 1.04x1.04x1 mm3, parallel imaging iPAT=2. Only one of the ten volunteers was additionally scanned with MT-prepared GRE. After taking into account effects due to T1, T2* and B1+, both M0 and the correction field can be directly estimated from the corrected signal values using a probabilistic framework for segmentation[4] as implemented in SPM8 (www.fil.ion.ucl.ac.uk/spm). The bias field was estimated only on brain tissue, but the bias field correction was calculated for and applied to the whole field-of-view. In the case of fixed tissue, water content was determined by referencing the M0 values obtained for the brain to the known water content of a reference probe immediately adjacent to the brain (same container) and containing a mixture of 80% H2O and 20% D2O. For the living brain, CSF was used as reference.

Results and discussion: Water content in fixed brains was found to have mean values of 72.9% for WM and 85.2% for GM with standard deviation (variability over 7 brains) of 2.6 and 2.4%, respectively. Water content in fixed brain tissue is thus a few percent units higher than *in vivo* (69.2% and 81.2% with SD of 1.9 and 1.2% over 10 volunteers, respectively) and of comparable constancy. Due to the small variability of this quantity, the difference is significant ($P<0.005$ for both WM and GM). In contrast, the T1 values were found to be greatly reduced compared to *in vivo* values, as reported before[5,6] and very variable. The same holds for the T1 contrast between WM and GM. The mean values (and SD) obtained here were: 375(148) ms for WM and 418(112) ms for GM, to be compared to 980 and 1500ms in vivo. Interestingly, the T2* values, although shortened, are found to be rather constant in fixed tissue: 34(6) ms for WM and 46(10) ms for GM, compared to 52 and 60ms for the living brain. The T2* contrast between WM and GM is quite pronounced, which is not the case *in vivo*. MTR was found to be variable from specimen to specimen and lower than *in vivo* by approximately a factor 2. A possible reason for this is the change in T1/ T2 (from 19 in vivo to 11 post mortem), thus reducing the influence of the direct effect[7] and the total MTR. Among the different other quantities and correlations investigated, only the whole-brain correlation between water content and T2* (corr coef. 0.66(0.14)), T1 and T2* (0.64(0.09)) and water content and MT measures were stable.

It might be assumed that the uncontrolled pre- and post mortem factors influencing tissue quality account for the variability of tissue properties; in any case, these factors appear to have a larger effect on T1 than on T2* and M0. Correlations between different parameters were investigated and found to be substantially different from the ones found in vivo. Most interestingly, the good correlation between T1 and water content reported many times in vivo is found to be quite limited for WM and diminishes substantially in fixed tissue. Instead, a good correlation between water content and T2* emerges, which was not found in vivo and the correlation between water content and MTR becomes stronger. In fixed as well as living tissue, water content and bound proton fraction are found to be well correlated. In conclusion, fixed brain tissue appears much more variable than living tissue, and factors other than myelin and water content appear to play a major role in the determination of relaxation times. This is perhaps not surprising given the important changes in the distribution of intra/extra cellular spaces and substances and given the large changes in membrane permeability that are induced by fixation.



Quantitative maps of a representative post mortem brain: a) water content in percent units; b) equilibrium magnetisation after MT (M0sat, percent units); c) T1 map [ms]; d) T1 after MT (T1sat) [ms]; e) T2* [ms]; f) MTR [pu] ; g) kfor [s-1]; h) fbound [a.u.] i) T1/T2*; j) T2*sat [ms].

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