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White matter hyperintensities analysis by diffusion tensor images obtained from *postmortem in cranium* whole brain tissue

Rafael E. Silva [a,b,*], Glaucia A.B. Santos [c,d], Ana T.D.L. Alho [c,d], Ricardo C. Neves [d], Luzia, L. Carreira [d], Lea T. Grinberg [d,e], Helmut Heinsen [b], Edson Amaro [a]

[a] *Departamento de Radiologia, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil*
[b] *Morphological Brain Research Unit, Universität Würzburg, Würzburg, Germany*
[c] *Hospital Israelita Albert Einstein, Instituto do Cérebro, São Paulo, Brazil*
[d] *Aging Brain Project, Department of Pathology, LIM-22, University of Sao Paulo Medical School, São Paulo, Brazil*
[e] *Memory and Aging Center, Department of Neurology and Pathology, University of California, San Francisco, San Francisco, USA*

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ABSTRACT

Purpose: In this paper we aimed at describing the quantifiable properties diffusion tensor images: fractional anisotropy (FA) and Apparent Diffusion Coefficient (ADC) at white matter hyperintensities (WMH) areas found *in situ* postmortem (PM) specimens. Our hypothesis is that the properties of WMH would be different from normal appearing white matter (NAWM) in the DTI images in postmortem.
Materials and methods: We analyzed PM MR images from 24 subjects (12 males; mean age: 67.2 ± 14.7 years) and *in vivo* (IV) MR images from 10 healthy volunteers (5 males; mean age: 62.3 ± 5.49 years). DTI processing was performed using the FSL platform; ROIs were placed at WMH and NAWM at FA and ADC maps.
Results: PM group presented FA values 26.75% lower at WMH than in NAWM. IV Mean FA in WMH was also reduced (17.76%) compared to NAWM. Average ADC from PM subjects was 6.89% higher at WMH than NAWM and 12.51% higher at WMH than NAWM at IV group.
Conclusions: We have demonstrated that *in situ postmortem* FA values at WMH are lower than NAWM, similar to *in vivo* data. This indicates that DTI obtained at a short postmortem interval from PM MRI could be used to understand *in vivo* MRI data.

1. Introduction

White matter hyperintensities (WMH) are common findings in T2-weighted Magnetic Resonance Images (MRI). These areas are frequently observed on healthy adults, reaching 94% of the elderly population and often described in medical reports as nonspecific findings [1–3]. Although several studies addressed this topic, there is no categorical definition of the etiology of these areas solely based on MRI characteristics [4,5]. Nevertheless, WMH incidence has important statistical association with clinical conditions, such as migraine, diabetes, cerebral ischemia [6,7]. Previous studies have shown WMH histological correlations including demyelination, metastases, gliosis and perivascular space increase [8–10]. Although WMH is strongly associated to a vascular etiology, it is also known that other mechanisms related to MRI and tissue properties can explain the finding in T2-weighted images [6,11,12].

These areas appear as small regions, with rounded shapes, and hyperintense on T2-weighted and fluid attenuation inversion recovery (FLAIR) MR images. The long T2 decay time indicates a localized increase in the content of hydrogen ions, most likely in water molecules. Structurally, WMH tissue substrates have been associated to decreased endothelial integrity, axonal regional degradations, such as loss of myelinated fibers, degradation of myelin sheaths and microvascular changes [5,10,13].

Regardless of underlying etiology, some authors associate the incidence of WMH to functional and cognitive changes. This hypothesis is presented with the correlation of these findings and signs of memory decline, intelligence, and autonomy, as well as psychiatric disorders such as depression [14–16]. However, there are no evidences establishing a direct relationship between WMH and neuropsychological performance at individual level.

Although there are various quantitative MRI acquisition techniques, none has been shown to unequivocally correlate to a specific histology feature – especially one that correlates with

* Correspondence to: Rafael Emídio da Silva, LIM-44, sala 4113, 4° andar, Faculdade de Medicina da USP, Av Dr. Arnaldo 455, São Paulo, SP, Brazil.
E-mail address: rafael.emidio@usp.br (R.E. Silva).

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WMH properties [17]. Over the past decade, MR quantitative techniques have been extensively used to investigate brain tissue properties. Particularly Diffusion Tensor Images (DTI), has been the main method used to investigate the properties of the white matter in the brain. DTI basic principles and properties can be found in detailed description a in the literature [23–28]. In short, DTI is based on patterns of molecular Brownian movement of water in biological tissues [24,29–31]. This property allows indirect evaluation of microscopic characteristics related to the direction of the white matter fibers, cell membranes, myelin, cytoskeleton and spatial arrangement of axons [32,33]. With DTI it is possible to quantify, in each voxel, parameters representing this properties – two of them are the most commonly investigated: fractional anisotropy (FA) and apparent diffusion coefficient (ADC). FA relates to directional properties of water diffusion in each voxel. Cell membranes and white matter tracts are one of the factors modulating this parameter, with values ranging from 0 (corresponding to fully isotropic diffusion) to 1.0 (indicating a completely anisotropic diffusion, *i.e.*, the water molecules only would diffuse in a single direction). ADC indicates the magnitude of the diffusivity of water in the tissue, related to overall molecular movement. Its value is reduced in the presence of spatial restrictions, such as viscosity and structural barriers present at extra- and intra-cellular levels [17,26,32,34].

DTI histological correlates in human brain are not fully determined (or, at least, only partially determined) in most clinical conditions – with a few exceptions, *i.e.*, for acute ischemia [18,23,33]. Nevertheless, DTI have significantly improved the accuracy of the images in relation to different changes detected in WMH [1,10]. Recent studies of white matter brain imaging techniques based on DTI showed promising results towards a better understanding of WMH etiology [31,35–39]. In most reports, authors mention DTI sensitivity to depict white matter organization in relation to myelin content and tissue spatial orientation. Reports using *postmortem* formalin fixated brain specimens showed that DTI parameters are altered in WMH and are correlated to gliosis in Alzheimer's disease as well as in healthy controls [40]. Even considering the relevance of this finding, the authors mention limitations of their method due to MR signal changes induced by the fixation process. Likewise, Gouw et al. reviewed the literature on histological-MRI correlations and showed that WMH were related to different levels of severity, as well as etiology in WMH in histology reports [1]. These authors conclude that, in general, *postmortem* studies showed that WMH are also heterogeneous in terms of histopathology. In this scenario, DTI data seems to be one of the best tools in terms of sensitivity to possible molecular changes in different histopathology entities.

On the other hand, methods allowing a precise spatial coincidence between histology and magnetic resonance images are still being developed. In this scenario, most scientific publications on MRI-histology correlations are based on images acquired from animal brains, or human fixated brain specimens [17–19]. The authors of these reports frequently mention limitations from species differences or tissue fixation artifacts (both in spatial distortion and MR tissue signal changes). Considerable signal abnormalities are described in gray and white matter MR brain images from fixated specimens (in general consisting of increased T1 and T2 relaxation times). These changes are most likely related to the chemicals used in the fixation processes, leading to the change of the water tissue content and proton density at tissue, and consequently alteration of the MR image signal [20–22]. Spatial distortions are also considerable due to fixation-induced brain swelling/shrinkage and can impair immunohistochemistry and stereological precise correlation protocols [23].

An alternative approach to the above mentioned limitation is to acquire MRI from *intracranial* brain specimens in a short *postmortem* interval. Although temperature and perfusion parameters do not emulate the conditions observed at *in vivo* subjects, MRI signal on images and spatial characteristics are remarkably comparable [7,10].

We have developed a workflow to acquisition and study *in situ postmortem* brain MRI data. This workflow has been applied to understand the histological properties of MRI signal in normal and diseased areas of human brain. In particular, the corresponding neuropathological alterations of areas detected by modern (non-classical) quantitative MR techniques currently used in the clinical setting could benefit from such approach [24,25].

In this paper we aimed at describing the quantifiable properties diffusion tensor images (FA and ADC) at WMH areas found *in situ* postmortem specimens. Our hypothesis is that the properties of WMH would be different from normal appearing white matter (NAWM) in the DTI images in postmortem. We also believe that such differences would be similar to *in vivo* data acquired in the same 3.0 T MR relatively short postmortem interval and absence of formalin fixation artifacts. In parallel, we investigated differences between *in situ* postmortem and *in vivo* data in order to evaluate potential clinical application of the method.

2. Methods

We analyzed MR *in situ postmortem* (PM) images from 24 subjects (12 males; 12 females) with mean age: 67.2 ± 14.7 years, and no previous history of neurological impairment. All postmortem specimens donated had an informed consent signed by a legal guardian and local Ethics Committee approved this study [41]. Data from these brain specimens were compared to *in vivo* (IV) MR images from 10 healthy volunteers (5 males; 5 females) with mean age: 62.3 ± 5.49 years. IV volunteers gave their written informed consent to participate on this study. Demographics from the participants are depicted in Table 1.

All images used in this study were acquired in the same 3.0 T MR system (Intera Achieva, Philips Medical Systems, Netherlands; operating at 80 mT/m gradients, with 100 mT/s rise time) with an eight channels head coil. In the PM group the images were acquired with the brain *in situ*, on a shortest viable *postmortem* mean interval (PMI): 14.4 ± 2.3 h. The corpses were laid on supine position, with arms beside the body and legs straight (protected by a purpose-build whole-body bag). No other manipulation was performed in the corpses before the MR examination. The body temperature was 27.22 ± 2.04 °C, measured by a digital thermometer with metal rod (Equitherm TM-879, Equitherm Com. And Serv., Brazil) inserted into the ear canal. Ambient temperature in the MRI exam room was kept within 20.0° ± 2 °C during all image data acquisition period.

All participants, both *in vivo* and *postmortem* subjects, underwent the same protocol, including the acquisition of the two datasets used in the analysis presented here: a) FLAIR: AC-PC orientated oblique axial slices, TR/TI/TE =9686.7/2800/130 ms,

Table 1
Descriptive analysis: *postmortem* and *in vivo* participants.

	Postmortem	***In vivo***
Participants (N, gender)	24(12 Males)	10 (5 Males)
Age (years)	67.2 ± 14.70	62.3 ± 5.49
Number of WMH ROIs	83	33
Number of NAWM ROIs	83	33
***Postmortem* Interval hrs.**	14.35 ± 02.26	–
Temperature	28.1 °C ± 3.21	37 °C ± 0.5

WMH: White matter hyperintensities; NAWM: Normal appearing white matter; ROI: Region of interest; °C: Celsius; ± : Standard deviation.