Forensic Science International 263 (2016) 139–146

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Diffusion tensor imaging of peripheral nerves in non-fixed post-mortem subjects

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ARTICLE INFO

Article history:
Received 26 November 2015
Received in revised form 10 February 2016
Accepted 1 April 2016
Available online 11 April 2016

Keywords:
Post-mortem imaging
Magnetic resonance imaging
Diffusion tensor imaging
Fiber tractography
Peripheral nerves

ABSTRACT

Purpose: While standard magnetic resonance imaging (MRI) sequences are increasingly employed in post-mortem (PM) examinations, more advanced techniques such as diffusion tensor imaging (DTI) remain unexplored in forensic sciences. Therefore, we studied the temporal stability and reproducibility of DTI and fiber tractography (FT) in non-fixed PM subjects. In addition, we investigated the lumbosacral nerves with PMDTI and compared their tissue characteristics to in vivo findings.

Methods: MRI data were acquired on a 1.5 T MRI scanner in seven PM subjects, consisting of six non-trauma deaths and one chronic trauma death, and in six living subjects. Inter-scan (within one session) and inter-session (between days) reproducibility of diffusion parameters, fractional anisotropy (FA), and mean diffusivity (MD), were evaluated for the lumbosacral nerves using Bland–Altman and Jones plots. Diffusion parameters in nerves L3–S2 were compared to living subjects using the non-parametric Mann–Whitney *U* test.

Results: Reproducibility of diffusion values of inter-scan 95% limits of agreement ranged from −0.058 to 0.062 for FA, and $(-0.037 \text{ to } 0.052) \times 10^{-3}$ mm^2/s for MD. For the inter-session this was −0.0423 to 0.0423, and $(-0.0442 \text{ to } 0.0442) \times 10^{-3}$ mm^2/s for FA, and MD, respectively. Although PM subjects showed approximately four-fold lower diffusivity values compared to living subjects, FT results were comparable. The chronic trauma case showed disorganization and asymmetry of the nerves.

Conclusion: We demonstrated that DTI was reproducible in characterizing nervous tissue properties and FT in reconstructing the architecture of lumbosacral nerves in PM subjects. We showed differences in diffusion values between PM and in vivo and showed the ability of PMDTI and FT to reconstruct nerve lesions in a chronic trauma case. We expect that PMDTI and FT may become valuable in identification and documentation of PM nerve trauma or pathologies in forensic sciences.

1. Introduction

Imaging techniques are becoming increasingly important in forensic examinations and may contribute to, or partially replace, conventional autopsy [1–4]. With computed tomography (CT) it is possible to identify fractures, pathologic gas development, large hemorrhages, and metal or other foreign bodies [5–9]. Magnetic resonance imaging (MRI) on the other hand is particularly valuable when soft tissue injuries or organ trauma need to be identified [5,8,10]. Regarding some of the more advanced MRI techniques, such as diffusion tensor imaging (DTI) [11–14], their potential value in forensic sciences remains unknown. DTI is an MRI technique which can measure the random movement of water molecules, known as Brownian motion [11]. In nervous tissue this movement is more pronounced along the nerve, so-called anisotropy [12]. Especially in the identification of peripheral nerves, DTI might be valuable, since nerves are difficult to dissect

Abbreviations: AD, axial diffusivity; DTI, diffusion tensor imaging; EPI, echo planar imaging; FA, fractional anisotropy; FOV, field of view; FT, fiber tractography; MD, mean diffusivity; MRI, magnetic resonance imaging; PM, post-mortem; PMDTI, post-mortem diffusion tensor imaging; PMMRI, post-mortem magnetic resonance imaging; RD, radial diffusivity; TSE, turbo spin echo.

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http://dx.doi.org/10.1016/j.forsciint.2016.04.001
0379-0738/

during conventional autopsy and are therefore typically neglected in forensic pathology. DTI can be used to characterize the structure of nervous tissue in detail and microstructural properties can be evaluated. DTI may serve as a valuable tool in investigations of accidents, homicides and other traumatic cases.

Important differences between post-mortem (PM) and living tissue should be considered. Shortly after death, processes such as autolysis occur. Combined with bacterial degradation, which facilitates tissue decomposition, nervous tissue will deteriorate [15]. Therefore, PMDTI has been performed predominantly on formaldehyde fixed tissue [16]. However, molecular diffusion characteristics are known to change markedly following the fixation process due to formation of intra- and inter-molecular cross-links [17]. Moreover, temperature regulation of PM subjects can be challenging during PMMRI, which can influence DTI results since water diffusion is highly temperature sensitive [18].

Previous PMDTI studies have focused on fixed human brains and demonstrated preserved diffusion anisotropy in nerves [15,17,19], allowing for fiber tractography (FT) and quantitative investigation of diffusion parameters such as fractional anisotropy (FA), which represents the degree in which diffusion is oriented in one direction, and mean diffusivity (MD), which is the average of all eigenvalues (the magnitude of diffusion in a direction). The FA and MD are based on the axial diffusivity (AD), which is the largest of the eigenvalues and in healthy nervous tissue diffusion is mostly represented along the nerve, and radial diffusivity (RD), which is the average of the second and third eigenvalue and in healthy nervous tissue diffusion is perpendicular to the nerve. Only a few studies have employed PMDTI in non-fixed human tissue, including the brain [20–23], brain trauma [24] and cardiac infarction [25]. The aim of this study was to demonstrate that PMDTI can be used for identification and quantification of peripheral nervous tissue in a reproducible way and to identify peripheral nerve trauma. Specifically, we investigated the lumbosacral nerves with PMDTI, because they already have been described in detail in several in vivo DTI studies [26–29], and we compared their tissue characteristics to in vivo findings. In addition, we studied the temporal stability and reproducibility of DTI and FT in non-fixed PM subjects.

2. Materials and methods

2.1. Subjects

In this study, seven non-fixed PM subjects with normal anatomy of the lower spine were included (five male, one female; median age of 46 years, range 30–55 years all non-trauma deaths, and one female trauma death; 35 years). PMMRI was performed 1–8 days after the estimated date of death. Specific subject information is listed in Table 1. Acquired data were included in four experiments (described below); data of subject 1–5 were included in experiment 1, data of subject 6 were included in experiment 2, data of subject 1–5 were included in experiment 3, and data of subject 7 were included in experiment 4. All subjects were cooled in the morgue at 4 °C before and after autopsy without fixative solution preparation. The routine autopsy was performed before PMMRI (except for experiment 2) and did not affect the lumbosacral area. Six healthy living subjects (mean age of 30 years, range 25–42 years) were included as reference subjects for experiment 3.

2.2. Data acquisition

MRI of the PM subjects was performed on a 1.5 T MRI scanner (Achieva; Philips Healthcare, Best, The Netherlands) using a 16-channel phased-array surface coil. PMDTI was performed with diffusion-weighted spin echo single-shot echo planar imaging (EPI) using the following parameters: TE = 82 ms, TR = 13,538 ms, SENSE factor = 2, number of excitations = 8, field of view (FOV) = 384 mm × 216 mm, matrix size = 128 × 72, 72, slice thickness = 3.0 mm, resulting in a voxel size of 3.0 mm × 3.0 mm × 3.0 mm, EPI factor = 35, *b*-values of 0 and 2000 s/mm^2, and 15 gradient directions. For three PM subjects the protocol was repeated two times within the scan session, and for four PM subjects it was repeated four times within the scan session, resulting in total scan times of 58 min and 1 h 56 min, respectively.

MRI of the six living subjects was performed on a 3 T MRI scanner (Achieva; Philips Healthcare, Best, The Netherlands) using a 16-channel phased-array surface coil. DTI was performed with diffusion-weighted spin echo single-shot EPI using the following parameters: TE = 58 ms, TR = 4209 ms, SENSE factor = 2, number of excitations = 2, FOV = 336 mm × 336 mm, matrix size = 112 × 112, slice thickness = 3.0 mm, resulting in a voxel size of 3.0 mm × 3.0 mm × 3.0 mm, EPI factor = 35, *b*-values of 0 and 800 s/mm^2, and 15 gradient directions. The total acquisition time was 4.5 min.

For anatomical reference, a 3D turbo spin echo (TSE) sequence was acquired based on the protocol described by [29] for both PM and living subjects.

2.3. Data analysis

DTI data from the PM and living subjects were processed identically, using the *ExploreDTI* diffusion MRI toolbox

Table 1
Subject specifications.

Identification number	Sex	Age in years	Approximate number of days between death and scan	Number of scans within one session	History	Cause of death	Extra information
1	Male	55	3	2	Alcohol abuse	Cardiac death	
2	Male	46	1	2	Depression, posttraumatic stress disorder	Gunshot through head	
3	Male	38	5	4	Drug abuse	Overdose methadone	
4	Male	30	5	4	Schizophrenia	Overdose of potassium tablets	
5	Male	46	3	4	Drug abuse	General physical decline	
6	Female	49	5	4	Schizophrenia, drug and alcohol abuse	Pancreatic cancer	Scanned three days before autopsy and once after autopsy
7	Female	35	2	2	Traffic accident during life, amputation right leg, injury to the lower lumbar vertebra years before death, incontinence and intestinal problems, hypoesthesia in the legs	Gunshot through head	