T2 relaxation time as a surrogate marker of liver fibrosis

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Introduction

Cirrhosis remains a major public health problem and disease-related complications were associated with nearly 40,000 deaths and more than 1.4 billion dollars spent on medical services in the US. The diagnosis, staging and quantification of fibrosis relies on liver biopsy [1], [2], however, liver biopsy is an invasive procedure with significant risks, including hemorrhage, infection and visceral perforation, and relatively high inter-observer variation interpretation and sampling errors in 25-45% cases. [1-3] It has long been noted that fibrotic livers demonstrate subjectively increased T2 signal intensity[4, 5]. We used this as a rationale to study retrospectively in patients with either Hepatitis B or C, all of whom had undergone random liver biopsy for fibrosis staging and had obtained a clinical MRI within 3 months of their biopsy, whether T2 correlated to liver fibrosis stage.

It was important to better understand and study whether any limitations existed with quantification of T2 with two echoes. We therefore prepared a phantom containing serial dilutions of superparamagnetic iron oxide magnetic nanoparticles (MNP) and compared the methodologies used in the clinical retrospective study, to more robust, established routines for quantifying T2 (e.g. Carr-Purcell-Meigboom-Gill pulse sequences). We then applied this methodology in an established animal model of liver fibrosis to test the hypothesis that T2 is a robust quantitative imaging biomarker of liver fibrosis.

Materials and Methods

***Phantom Study*:** Our phantoms consisted of one cc of MNP (R2 – 49 sec $^{-1}$) (1 mg Fe/ml) diluted with the following fractionations in saline (**1:250, 1:500, 1:1000**) and placed in 50cc Eppendorf (Fischer Scientific) tubes. MRI was performed in a 1.5T Siemens Avanto with TIM technology using a 4 channel head coil. All imaging was performed with asymmetric FOV (16 x 14cm) ; (512 x 409); 4mm slice thickness, interleaved with no gap. The CPMG sequences were performed with TR 5000msec. and TE (22 msec. in 22 msec. intervals). The TSE sequences were performed with effective TE of 22 and 86msec., echo train length of 7. T2 was fit using a mono-exponential fit (Osirix ®).

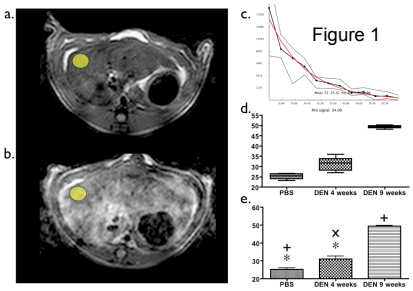

Figure 1

Rat model of liver fibrosis: All animals received humane care and were maintained in accordance with the institutional guidelines of the MGH Subcommittee on Research Animal Care. Male Wistar rats received weekly IP injections of diethylnitrosamine (DEN) at 100 mg/kg or vehicle control (PBS) for either 5 or 8 weeks. Preliminary results with this model demonstrated fibrosis after 5 weeks and cirrhosis after 8 weeks of DEN treatment. Rats were imaged at 6 and 9 weeks after a one-week washout period to eliminate acute effects of DEN. After imaging, the animals were sacrificed and liver was then sectioned and fixed in phosphate-buffered 10% formalin for histological analysis by Masson's trichrome staining. MRI was performed at 4.7T on a Bruker imaging system (Pharmascan, Karlsruhe, Germany). Animals were anesthetized during imaging with 1-1.5% inhaled Isofluorane, and monitored during imaging with respiratory monitoring. Imaging protocols included multi-slice multiecho (MSME) T2-weighted imaging for T2 quantification. The following parameters were utilized: Flip = 90^0; Matrix (128 x 64); TR = 2500msec.; TE = 8.6- 137msec in 16 echoes at 8.6msec steps; field of view (FOV) = 4.24 x 2.12 cm, slice thickness = 1mm.

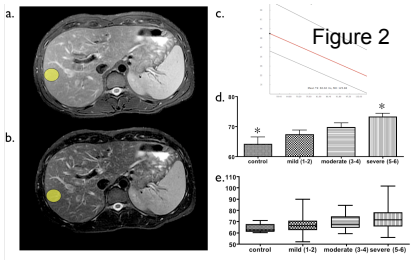

Figure 2

Retrospective human study: This retrospective study was HIPPA compliant study, approved by MGH IRB and patient informed consent was waived. N=123 patients with chronic active hepatitis C in whom a random liver biopsy was performed within 6 months during the period 07/1999-1/2010 participated. The average time between MRI and biopsy was -8days (range: -165 to 177). All biopsies were staged histologically (Ishak(0-6)) and grouped into control (stage (0) n=0); mild (stage (1-2) n=30), moderate (stage (3-4), n=27), severe (stage (5-6), n=66). MR imaging was performed with a phased array body coil (8 channel) on either Siemens 1.5T Avanto/Allegra or GE Excite using RARE equivalent pulse sequences with dual TE values (46-99msec. and 84-177msec.) and TR 2000-3500msec. T2 was fit from ROI data in the same representative area of right lobe of the liver on both echoes and fitting with a two-point fit (MS Excel, Seattle, WA). Statistical analysis included one way ANOVA and student's unpaired t-test between individual groups.

Results

Our phantom results demonstrated close agreement between mean T2 values in all MNP dilutions (**1:250, 1:500, 1:1000**) comparing TSE (53 +/- 2, 103 +/- 2, 188 +/- 6) and 12 echo CPMG (52 +/- 3, 102 +/- 2, 192 +/- 2). Figure 1 demonstrates examples of a normal PBS rat (1a), and cirrhotic (DEN 8) rat (1b). T2 was quantified with a 16 echo multi-slice multi echo turbo spin echo sequence at 4.7T. ROI were placed within the right lobe excluding major vessels and areas with artifact (yellow elliptical ROI). T2 was then quantified in all groups and included the following: a) n=4 PBS normal control rats; b) n=5 DEN rats treated for 5 weeks (moderate (Ishak 4) fibrosis); and c) n=4 DEN rats treated for 8 weeks (Ishak 6 cirrhosis). Quantitative analysis demonstrated a monotonic increase in T2 between each rat subgroup ((PBS (25.2 +/- 0.8), DEN 5week(31.1 +/- 1.5), and DEN 9 week (49.4 +/- 0.4)) msec, and confirmed our hypothesis. Furthermore, there was a statistically significant difference between all groups (ANOVA - $p<0.0001$), and also between each subgroup (unpaired t-test, ($p<0.001$ (PBS:DEN 9; DEN 4:DEN 9)), ($p<0.05$ (PBS:DEN 4)).

Figure 2 (a,b) demonstrates T2 weighted MR images of a patient with chronic active hepatitis C (TE 60 (a), 120 (b)). ROI (yellow ellipse) were taken within the right lobe of the liver avoiding major vessels, attempting to be within the area most often biopsied and free from phase encoding artifact. T2 was quantified by using a two point fit (Figure 2c). Figure 2d demonstrates the values of the absolute T2 measurements (control 65.4 +/- 2.9msec.; mild 66.7 +/- 1.9msec; moderate 71.6 +/- 1.7msec; severe 72.4 +/- 1.4msec), which demonstrated low standard error (~2.9msec). Furthermore, there was a monotonically increasing mean T2 value with increasing degree of histologic fibrosis. There was a statistically significant difference between degrees of mild vs. severe fibrosis ($p<0.05$) with results approaching statistical significance amongst all groups by ANOVA ($p<0.1$).

DISCUSSION

Our retrospective analysis demonstrates that there is a monotonically increasing T2 value with increasing fibrosis stage, which expands on our previous results [6], and as has been noted in other imaging methods, most notable (MRE[7-9], and DWI [10]). One potential criticism with this retrospective analysis is the high variance and difficulty in quantifying T2 with only two values. Our phantom data demonstrate, however, that there is close agreement in absolute T2 values when comparing a 2 point fit from RARE/TSE data as compared to conventional T2 quantification with multi-echo CPMG data in T2 values that are relevant for the liver. Furthermore, our animal results corroborate our human studies with markedly lower variance. In summary, this data corroborates a possible quantifiable inflammatory infiltrate that can be seen in patients with liver fibrosis, especially in patients with chronic hepatitis C infection. Furthermore, our data demonstrate that there was a statistically significant difference in absolute T2-relaxation time between mild degrees of fibrosis as compared to severe fibrosis, which imply that T2 may be a surrogate marker of liver fibrosis.

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