Quantitative Analysis of Diffusion Tensor Imaging in Chronic Hepatitis in Rats

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Abstract

Background
Diffusion tensor imaging (DTI) is mainly used for detecting white matter fiber in the brain. From this, DTI has been applied to assess fiber in liver disorders by prior studies. But non-sufficient data has been obtained if DTI could be used for exactly staging chronic hepatitis. This study is to assess the value of DTI for staging of liver fibrosis (F), necroinflammatory activity (A), and steatosis (S) of chronic hepatitis in rats.

Methods
Seventy male Sprague-Dawley rats were divided into control group (n = 10) and experimental group (n = 60). The rat models of chronic hepatitis were established by abdominal subcutaneous injections of 40% CCl_4. All rats underwent 3.0T MRI. ROIs were placed on DTI to estimate MR parameters (rADC value and FA value). Histopathology was the reference standard. Multiple linear regression was used to analyze the association between MR parameters and pathology. The differences in rADC value and FA value among pathological stages were evaluated by MANOVA or ANOVA. LSD was used to test the differences between each two groups. ROC analysis was performed.

Results
The numbers of each pathology were as follows: F0(n = 15), F1(n = 11), F2(n = 6), F3(n = 9), F4(n = 6); A0(n = 8), A1(n = 16), A2(n = 16), A3(n = 7); S0(n = 10), S1(n = 7), S2(n = 3), S3(n = 11), S4(n = 16).

The rADC value had a negative correlation with liver fibrosis ($r = -0.392, P = 0.008$) and inflammation ($r = -0.359, P = 0.015$). FA value had a positive correlation with fibrosis ($r = 0.409, P = 0.005$).

Significant differences were found in FA value between F4 and F0 ~ F3 ($P = 0.03$), while no significant differences among F0 ~ F3 were found ($P > 0.05$). AUC of FA value in differentiating F4 from F0 ~ F3 was 0.909 ($p < 0.001$) with 83.3% Sensitivity, 85.4% specificity when the FA value was at the cut-off of $588.089 \times 10^{-6}$mm$^2$/s.

Conclusion
FA value for DTI can distinguish early cirrhosis from normal, mild and moderate liver fibrosis.

1. Background
Chronic hepatitis is a typical chronic diffuse liver disease which caused by many factors (1). The basic pathological changes of chronic hepatitis include hepatic inflammation, liver fibrosis and fatty
infiltration and the disease can further develop into cirrhosis, even liver cancer and liver failure (2, 3). Studies (4) have shown that early chronic hepatitis is a dynamic and reversible lesion. Early diagnosis and accurate staging of chronic hepatitis has a clinical significance in evaluating the severity and progress of the disease. Percutaneous liver biopsy is considered as the gold standard, but patients always reject this an invasive technique for they usually have no symptom (5).

Several noninvasive methods have been put forward to chronic hepatitis, among which the most promising ones are ultrasound elastography and magnetic resonance elastography, but the former is inadequate or unavailable in obese and abdominal dropsy patients and the other one is expensive. DTI is a mature magnetic resonance imaging (MRI) sequence developed on the basis of diffusion weighted imaging (DWI). Compared with the undirectional or three orthogonal directional DWI, DTI quantifies the diffusivity of water molecules by using six or more different directions of diffusion sensitive gradients, traces the fiber bundle shape, visually reveals the microstructure characteristics of biological tissues. DTI sequence achieves the average diffusion coefficient (rADC) image, fractional anisotropy (FA) image, relative anisotropy image and their corresponding values. Among which, rADC and FA value are widely used (6).

DTI is mainly used for detecting white matter fiber nervous system (7). From this, some studies (8-11) have applied DTI for chronic hepatitis considering that fibrosis always emerges in liver damage. Previous studies (10, 11) showed that the CCl4-induced liver fibrosis animal model was a mature technique, and it could mirror pathophysiologic processes of fibrogenesis in human (12). However, non-sufficient data has been obtained. Our study adopted DTI to investigate rADC and FA values of chronic hepatitis in rats.

2. Methods
2.1 Establishment of chronic hepatitis model in rats
This study was approved by the Animal Ethics Committee of the Southwest Medical University.

Seventy male Sprague-Dawley rats aged 6–7 weeks and weighted 150–200 g were randomly divided into control group (n = 10) and experimental group(n = 60). The rats were purchased from Animal Experimental Center of Southwest Medical University. Chronic hepatitis model was induced in rats by
abdominal subcutaneous injection of 40%CCl₄ suspension (99.9% carbon tetrachloride: vegetable oil = 4:6) at a dose of 0.3 ml/100 g twice a week. The rats in the control group were injected with 0.9% sodium chloride at the same dose and in the same way. The animals were raised under the standard conditions, freely having food and water.

2.2 MR imaging
Five weeks after injecting CCl₄, 6 to 10 rats for test group and 1 or 2 rats for control group were randomly selected for MRI scan every week. The rats were supinely fixed on boards under anaesthesia during scanning by intraperitoneal injection of 1% pentobarbital sodium at the dose of 0.5 ml/100 g.

The MR exams were performed on a 3.0T MRI scanner(Achieva 3.0 T, Philips, Netherlands) using 8-channel knee coils. Spin-echo echo-planar imaging diffusion tensor imaging sequence; two b values (0 and 800 s/mm²); 15 diffusion gradient directions; TR, 3907 ms; TE, 86 ms; FOV, 100mm × 100 mm, thickness, 2.0 mm; NSA, 3; matrix, 240.

2.3 Image analysis
Two radiologists who were blinded to the pathological results used post-processing workstation(Philips Extended MR WorkSpace 2.6.3.4)to generate functional imaging maps(rADC image and FA image) and measuring the quantitative indicators of region of interests (ROIs).Three circular ROIs per slice ranged 5 mm² ~ 10 mm² were placed on two consecutive slices of DTI images of b = 0 s/mm² and then were copied to the same slices of rADC images and FA images (Fig. 1). Mean values of the six ROIs measured by the two radiologists respectively were estimated. Average value of the two radiologists measured for each specimen was taken as the final measurement. Care was taken to avoid large vessels and the edge of ROIs was at least 3 mm away from the border of the liver.

2.4 Histopathological evaluation
The rats were sacrificed by cervical dislocation for pathological evaluation immediately after MRI Scan. Hematoxylin and eosin staining and Masson staining were performed. Stage of hepatic fibrosis (F) and necroinflammatory activity (A) were evaluated according to the METAVIR scoring system (13). Steatosis(S) was depended on the percentage of liver cells containing fat droplets as follows:
S0(0%-5%), S1 (6%-30%), S2 (31%-50%), S3 (51%-75%), and S4 (> 75%). The liver sections were assessed by two pathologists who didn’t know the radiologic outcome.

2.5 Statistical analysis
The intraclass correlation coefficient (ICC) was performed to evaluate the reliability of values measured by the two radiologists. Multiple linear regression (Enter model) was used to analyze the relationship between MR parameters (rADC value and FA value) and pathological stage (necroinflammation, hepatic fibrosis and steatosis) respectively. The differences in magnetic resonance parameters among pathological stages were evaluated by multi factor analysis of variance (MANOVA) or one-way analysis of variance (ANOVA). The least significant difference (LSD) method was used to test the differences between each two groups. To evaluate the diagnostic performance of FA value for the assessment of fibrosis stage, receiver operating characteristic (ROC) curve was performed. A P value less than 0.05 was thought to be statistically significant.

3. Result
3.1 General situation of animal models and pathologic results
Sixteen rats died in the experimental group during inducing model. Five specimens in experimental group and one specimen in control group which had low signal-to-noise ratio were eliminated despite taking measures to reduce artifacts. Finally, there were 47 specimens available including 8(8/10) control group rats and 39(39/60) chronic hepatitis rats. The statistical data was as follows: F0(n = 15), F1(n = 11), F2(n = 6), F3(n = 9), F4(n = 6); A0(n = 8), A1(n = 16), A2(n = 16), A3(n = 7); S0(n = 10), S1(n = 7), S2(n = 3), S3(n = 11), S4(n = 16). All the data were summarized in Table 1.
Table 1
Distribution of rADC value and FA value in pathology of the liver

| Pathological stage | number | rADC (×10⁻⁶mm²/s) | FA (×10⁻⁶mm²/s) |
|--------------------|--------|--------------------|-----------------|
| A0F0S0             | 8      | 923.008 ± 69.899   | 505.421 ± 34.550|
| A1F0S1             | 2      | 885.900 ± 19.351   | 489.075 ± 44.182|
| A1F0S4             | 1      | 1167.383           | 372.433         |
| A1F1S1             | 2      | 941.417 ± 128.057  | 455.658 ± 118.688|
| A1F1S4             | 4      | 852.388 ± 150.181  | 553.717 ± 99.250|
| A1F2S0             | 1      | 875.067            | 582.250         |
| A1F2S3             | 2      | 760.892 ± 30.205   | 526.508 ± 55.425|
| A1F2S4             | 2      | 839.900 ± 47.588   | 526.508 ± 55.425|
| A1F3S0             | 1      | 1167.383           | 372.433         |
| A1F3S3             | 2      | 852.233            | 405.000         |
| A2F0S4             | 1      | 683.100            | 323.892 ± 30.205|
| A2F1S3             | 1      | 774.908 ± 88.565   | 535.308 ± 140.184|
| A2F1S4             | 2      | 795.483 ± 128.615  | 465.306 ± 181.995|
| A2F3S0             | 1      | 866.550            | 582.167         |
| A2F3S3             | 1      | 637.583            | 598.350         |
| A2F3S4             | 2      | 818.750 ± 26.729   | 494.017 ± 73.044|
| A2F4S0             | 1      | 875.067            | 582.167         |
| A2F4S1             | 2      | 651.425 ± 28.555   | 731.483 ± 21.920|
| A2F4S2             | 2      | 701.783            | 759.617         |
| A2F4S3             | 2      | 774.775 ± 94.599   | 576.917 ± 26.328|
| A3F0S3             | 1      | 774.975 ± 63.062   | 530.283 ± 58.266|
| A3F0S4             | 1      | 763.033            | 606.250         |
| A3F1S4             | 1      | 826.183            | 525.267         |
| A3F2S4             | 1      | 736.933            | 544.250         |
| A3F3S3             | 2      | 804.083            | 545.117         |
| A3F3S4             | 1      | 743.350            | 634.433         |
| A3F4S0             | 1      | 803.050            | 566.138         |

Abbreviations: A, necroinflammatory activity; F, fibrosis; S, steatosis; rADC, average diffusion coefficient; FA, fractional anisotropy.

3.2 MRI quantitative indicators

The ICC of rADC value was 0.852 (P<0.001), and FA value was 0.922 (P<0.001). High measurement repeatability between the two observers speaks to the clinical feasibility of this method.

The result of Multiple linear regression analysis was presented in Table 2. The rADC value was correlated with fibrosis (r=-0.392, P = 0.008) and necroinflammatory activity (r=-0.359, P = 0.015), but not with steatosis (P = 0.452). FA value was related to fibrosis degree(r = 0.409, P = 0.005), but not with inflammatory activity(P = 0.236) and steatosis (P = 0.115). Table 3 showed the rADC value and FA value of different stages of liver fibrosis, indicating that the rADC value decreased with the severity of liver fibrosis, while the FA value increased.

Table 2
Results of the regression analysis between quantitative indexes of DTI and pathology

| Pathologic staging | rADC value | FA value |
|--------------------|------------|----------|
|                    | B          | r        | P        | B          | r        | P        |
| A                  | 48.243     | -0.359   | 0.015*   | 20.924     | 0.180    | 0.236    |
| F                  | 29.365     | -0.392   | 0.008*   | 28.175     | 0.409    | 0.005*   |
| S                  | 7.952      | 0.115    | 0.452    | -15.368    | -0.238   | 0.115    |

Abbreviations: A, necroinflammatory activity; F, fibrosis; S, steatosis; rADC, average diffusion coefficient; FA, fractional anisotropy. *Significant at P < 0.05. B value, unstandardized coefficient. r value, partial correlation coefficient.
Table 3
Results of the quantitative analysis of rADC and FA value in according to the fibrotic stage

| Fibrotic stage | rADC value($\times 10^{-6}$mm²/s) | FA value($\times 10^{-6}$mm²/s) |
|----------------|----------------------------------|-------------------------------|
| F0             | 899.231 ± 109.210                | 497.718 ± 61.249              |
| F1             | 835.283 ± 129.938               | 519.286 ± 123.599             |
| F2             | 802.264 ± 61.647               | 531.164 ± 52.087              |
| F3             | 786.091 ± 70.868               | 542.944 ± 66.228              |
| F4             | 716.256 ± 72.060               | 668.475 ± 84.078              |

Abbreviations: F, fibrosis; rADC, average diffusion coefficient; FA, fractional anisotropy.

By the means of MANOVA, no significant differences were found among stages of fibrosis ($F = 1.250$, $P = 0.309$) and inflammatory activity ($F = 1.487$, $P = 0.236$) for rADC value. Analyzed by ANOVA, FA value among different fibrosis groups was significant different ($F = 4.750$, $P = 0.03$). There was a significant difference in FA value between F4 and F0 ~ F3 ($P < 0.05$), while no significant differences among F0 ~ F3 were found ($P > 0.05$). The area under the ROC curve (AUC) of FA value in differentiating F4 from F0 ~ F3 was 0.909($p < 0.001$) at the cut-off of 588.089($\times 10^{-6}$mm²/s), with 83.3% Sensitivity, 85.4% specificity.

4. Discussion
Our study found that the rADC value was negatively related to hepatic fibrosis and necroinflammatory activity, but not related to steatosis. FA value had a positive correlation with fibrosis degree, but had no correlation with necroinflammatory activity and steatosis. The FA value of F4 was statistically different from F0 ~ F3, while there were no significant differences among F0 ~ F3, suggesting that liver FA value can distinguish early cirrhosis(F4), but it had little significance in differentiating normal, mild and moderate liver fibrosis(F0 ~ F3).

DTI can effectively detect the free diffusion rate of water molecules with different structures in vivo and can more accurately reflect the changes in the direction of water molecules dispersion, providing both functional and microstructural information in liver by means of water diffusivity and diffusion anisotropy quantitation, and thus may contribute to evaluating liver fibrosis(11). The reduction of rADC value with fibrosis observed in our study was in accordance with most prior researches(14–16).

However, both our and previous studies (9, 15) found that rADC value lacked the ability of differentiating the fibrotic grades. The relationship between FA value and liver fibrosis and the evaluation of FA value for fibrosis staging were reported differently. Cheung et al(11) found that FA value of rats at 2 weeks after CCl₄ insult was both significantly lower than that before and 4 weeks
after the insult, while FA value at 4 weeks after CCl₄ insult was not significantly different from that before insult, which suggesting that FA value can reveal the progression of liver fibrosis, especially early cirrhosis. Another animal research used C57BL/6 mice (10) reported that FA was negatively correlated with hepatic fibrosis and the model group(n = 20) had a lower FA than control group(n = 16). But the data in this study was relatively small (F1 = 4, F2 = 11, F3 = 5) and missing value contained in F4. On the contrary, Tosun M et al (9) found that FA values showed a trend toward higher values with increasing fibrotic stage, but there were no statistically significant differences between the FA values at different fibrotic stages. Our study also found a positive correlation between FA value and fibrosis degree. Meanwhile, our study showed FA value of F4(early cirrhosis) was significantly different from F0 ~ F3. Liver cirrhosis is the end stage of liver fibrosis which has small chance to reverse and high risk of develop into complications and hepatocellular carcinoma. Yet radiologists can’t diagnose early cirrhosis rely on conventional medical imaging because the morphological changes are not obvious. Our study results found that FA value of DTI could distinguish early cirrhosis, which may help physicians to take measures early. The explanation for our study results probably was that with the increasing degree of liver fibrosis, the free movement of water molecules in the liver was affected by the presence of the fiber composition, and the movement direction tended to be consistent or opposite, which increased the FA value. FA value of F0-F3 was not significantly different because that in the early stages of liver fibrosis, the distribution of collagen fibers was not regular and directional, which resulted in the restricted diffusion of water molecules in all directions, leading to the direction of the main axis of water molecules movement less obvious. As a result, the FA value changed not so remarkably. With the progress of fibrosis, the fibrous bundles increased, joined into strips, flakes, and rearranged, which made the main axis of water molecule diffusion more obvious so the FA value was increased significantly in F4(early cirrhosis).

There was limited data about the relationship between liver necroinflammatory grade and DTI measurements. In general, studies (8, 9) found that liver ADC values was inversely correlated with inflammation. But the rADC cannot discriminate different inflammation grades. Both our and Tosun M’s (9) study demonstrated that FA value was not related to inflammation grades. The explanation for
rADC decreased with increasing inflammation grades may be a large number of inflammatory cells and factors helped to restrict the free movement ability of water molecule. But it didn’t influence the movement direction of water molecule, so the FA value had no means with inflammation. Because the clinical therapy depends on the fibrotic stage and inflammatory grade, in prior studies there were mainly about DTI for liver fibrosis and inflammation (8, 9). But some scholars emphasized that ADC (17-20) and FA value (10) in the liver need to be carefully interpreted in the presence of hepatic steatosis. Besheer T (17) demonstrated that hepatic steatosis should always be considered when assessing hepatic fibrosis and their study found that detected hepatic steatosis would underestimate ADC value in patients with chronic hepatitis C. Accordingly, our study took steatosis into consideration. However, our study results did not find the relationship between steatosis and both rADC and FA value, which disaccorded with some of previous research (17, 20-22). There were other studies (13, 23) demonstrated no significant relationship between ADC value and steatosis, which were similar to ours. The inconsistent relationship reported by researchers between MR measurements and steatosis cannot be accurately explained. Maybe different MR machines and parameters or different group standards could affect the results.

There were limitations in our study. First, the main deficiency was that the echo-planar imaging sequence of DTI had a lower signal-to-noise ratio and artifactual interference. Second, the distribution of pathological groups was uneven. Different rats had individual difference in sensitivity to the induction of chronic hepatitis after injection of the same dose of drugs, which made the number of some groups relatively small. Future studies are needed to adopt a high signal-to-noise ratio sequence. Chronic liver disease patients who were performed hepatectomy could enrolled in future study.

5. Conclusion
Our experiment showed that the rADC value of DWI sequence was inversely related to hepatic fibrosis and inflammation and FA value had a positive correlation with fibrosis degree. FA value had a high diagnostic accuracy in differentiating early cirrhosis, so FA value would be a potential marker for diagnose of liver cirrhosis.
Abbreviations

DTI: diffusion tensor imaging;

F: liver fibrosis;

A: necroinflammatory activity;

S: steatosis

MRI: magnetic resonance imaging

DWI: diffusion weighted imaging;

rADC: average diffusion coefficient;

FA: fractional anisotropy;

ROIs: region of interests;

ICC: intraclass correlation coefficient;

MANOVA: multi factor analysis of variance;

ANOVA: one-way analysis of variance;

LSD: least significant difference;

ROC: receiver operating characteristic curve

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving animals were in accordance with Guiding Principles for the Care and Use of Animals and was approved by the Animal Ethics Committee of the Southwest Medical University of Luzhou, Sichuan, China and the laboratory animal production licenses were SCXK (Chuan) 2013-17, SCXK (Chuan) 2013-181 and SCXK (Chuan) 2013-065.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request at shujiannc@163.com.

Competing interests
All the authors declare no competing interests.

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Authors' contributions

Contribution to conception and design: HMP, LX, WXF and SJ;
Contribution to data acquisition and interpretation: HMP, LX and WXF;
Contribution to performance of all statistical analyses: HMP and LX;
Contribution to drafting of manuscript: HMP and LX;
Contribution to critical revision of manuscript: SJ.

HMP and LX had materially participated in the research and made the equal contribution, so they all ranked as the co-first authors.

All authors read and approved the final manuscript.

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Figures

![Figure 1](image)

Figure 1

The rADC and FA value calculation A Three circular ROIs ranging 5mm2~10mm2 were placed on the DTI images of b=0s/mm2. B ROIs were copied to the rADC image and the computer calculated the rADC values. C ROIs were copied to the FA image and the computer calculated the FA values.

Supplementary Files
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