Comparative Study of Imaging and Pathology of Sacroiliac Joint in Normal Rats Based on IVIM-DWI and DCE-MRI

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Abstract

Background: There are few reports describing the relationship between functional MRI findings and pathology of normal sacroiliac joint (SIJ). Furthermore, the pathology of SIJ is difficult to access, so animal models have special value.

Aims: We undertook this study to explore the changes of parameters of Intravoxel Incoherent Motion Diffusion Weighted Imaging (IVIM-DWI), Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) and compare them with pathology in the SIJ of normal rats in different ages.

Methods: Thirty 7 week-old male wistar rats were included in the study. The parameters of IVIM-DWI and DCE-MRI in bone marrow and the joint space of SIJ were measured respectively at 8, 13, 18, 23, 28 and 33 weeks, then histological specimen of the SIJ were examined with light microscopy. One-way ANOVA were used for statistical analysis.

Results: The values of D in the sacral and iliac bone marrow of normal rats in different age groups decreased with the increase of age, and ANOVA indicated a significant difference in D values among different age groups (P < 0.005). The normal values of D*, f, Fenh(%), Senh(%/s) in the sacral bone marrow, the iliac bone marrow and in the joint space in SIJ of normal rats were obtained. In six groups of rats with different weeks of age, the pathology of the SIJ surface was smooth and clear, the cartilage cells were intact, and no thickening or pannus formation was found.

Conclusions: The range of IVIM-DWI and DCE-MRI parameters of the sacral and iliac bone marrow and of the synovial area of the joint space in normal rats were obtained. It provides a basis for further clinical study of SIJ lesions.

Background

The SIJ is an auricular shaped joint known to transfer forces between the lumbar spine and lower limbs. The auricular joint surface connects sacral and the iliac bones and is surrounded by strong ligaments[1,2]. Functionally speaking, the SIJ is a joint with limited motion capabilities. So, it's main function is to maintain body stability. Besides, sacroiliitis is considered to be an important criterium for the diagnosis of ankylosing spondylitis and is the most important factor in different ankylosing spondylitis stages[3]. To understand the pathologies arising in the SIJ, it is very important to
understand the normal anatomy, pathology and imaging characteristics of the normal SIJ. Previous articles have focused on some parts of the descriptive anatomy, diagnosis, and treatment of SIJ pathologies[4,5]. Other studies have also emerged on the biomechanics of the SIJ, in terms of the structural relationships of the joint with surrounding tissues[6,7]. In recent years, there has been a profound study on the application of new imaging techniques for early arthritis[8], but there is a lack of comparative studies regarding the normal SIJ. Some scholars have used male corpses for the pathological anatomy and related MR examinations of the SIJ[9]. These studies cannot reflect the physiological condition of a normal and abnormal SIJ in the living state and cannot be used to extract quantitative imaging parameters from. In summary, there are few reports describing the relationship between functional MRI findings and pathology of normal SIJ. Furthermore, the pathology of the SIJ is difficult to access, so animal models have special value in providing opportunities for quantitative parameter extraction on imaging.

In this study, we hypothesized that there are relationships between the IVIM-DWI and DCE-MRI parameters on the one hand and the pathology results of the normal SIJ on the other hand. To test this hypothesis, we measured MR parameters of IVIM-DWI, DCE-MRI in rats of different age groups and compared them with pathological results. We hope that our study can lay a foundation for our further study of sacroiliitis in rat models.

Methods

2.1 Research objects

Thirty male wistar rats were obtained from Experimental Animal breeding Co., Ltd.(China, Jinan). The rats were 7 weeks old and weighed 170-200g. All rats were acclimatized in a Specific Pathogen Free(SPF) environment for one week before the experiment. At 8, 13, 18, 23, 28 and 33 weeks, 5 rats were randomly selected for MRI examination under anaesthesia (3ml of Urethane intraperitoneal injection), then euthanized and sent for pathology acquisition. Urethane was purchased from Shanghai Shanpu Chemical Co., Ltd. A 0.2g/mL solution of urethane was prepared using saline (total of 10ml/kg) with intraperitoneal injection. The study was approved by the Institutional Animal Care and Use Committee and was performed in accordance with the National Institutes of Health guidelines for
the use of laboratory animals.

2.2 MR Imaging Techniques

All MR imaging was performed using a 3.0-T MR (GE discovery MR750) and utilizing a matched eight-channel animal coil (Wankang Medical Technology Co., Ltd, China). Four standard MR sequences were performed. (A) Axial T2 fat saturated (FS) images [echo time (TE)/repetition time (TR), 96.1 ms/3000 ms; echo train length, 16]; (B) Coronal T1 FS fast spin echo (FSE) (TE/TR, 13.5 ms/500 ms; echo train length, 3); (C) DCE-MRI: fat saturated contrast enhanced T1 images with liver acquisition with volume acceleration (LAVA) sequence, repetition time/echo time (TR/TE) 5.6/1.9 ms, slice thickness 2.0 mm, matrix 128×128, FOV 200 mm×160 mm. A total of 80 phases were acquired, with a spatial resolution of 2.0 mm². 1 ml of gadopentetate dimeglumine contrast agent (BeiLu Pharmaceutical Co., Ltd, Beijing, China) was administered intravenously (tail vein) at a rate of 0.1 ml/s, followed by a 2 ml saline (0.9%, Shandong Qidu Pharmaceutical Co., Ltd) flush by hand. The concentration of gadopentetate dimeglumine is 0.5 g/ml. After the acquisition of seven baseline dynamic scans, 960 images were collected totally with 80 phases for approximately 5 minutes of scanning; (D) IVIM-DWI: repetition time/echo time (TR/TE) 4000/66.8 ms, slice thickness 3.0 mm, matrix 64×64, FOV 140 mm×112 mm, spatial resolution 3.8 mm². A total of 12 b-values were used: 10, 20, 30, 50, 80, 100, 200, 300, 600, 800, 1000, 1500 s/mm². Function tool software of GE MR Advantage Workstation 4.6 was used to perform the measurements of IVIM-DWI and DCE-MRI.

2.3 Image Analysis

2.3.1 Analysis of IVIM Parameters

The DWI signal follows the biexponential model to calculate the signal attenuation IVIM, as [10]:

\[ \frac{S(b)}{S_0} = (1-f) \exp(-bD) + f \exp[-b \times (D^* + Dblood)] \]

Where \( S_b \) is the signal intensity in the pixel with diffusion gradient \( b \), \( S_0 \) is the signal intensity without diffusion gradient, \( D \times 10^{-4} \) mm²/s is the water diffusion coefficient in the tissue, \( f \) is the perfusion fraction related to microcirculation (flowing blood fraction) and \( D^* \times 10^{-4} \) mm²/s is the pseudo-diffusion coefficient which represents perfusion-related diffusion.
2.3.2 Analysis of DCE-MRI Parameters

For DCE-MRI analysis, all data were quantitatively analyzed using image processing software. By manually drawing different regions of interest (ROIs), we obtained time-intensity curves (TICs).

Enhancement factor $F_{ehn} (%) = \frac{(SI_{max} - SI_0)}{SI_0} \times 100\%$

Enhancement slope $S_{enh} (%/s) = \frac{(SI_{max} - SI_0)}{100 / (SI_0 \times T_{max})}$

where $SI_0$ (baseline Signal Intensity before contrast injection) approaches the $SI_{max}$ (maximum Signal Intensity) exponentially in time. $SI_{max}$ is determined as the maximum SI during the DCE-MRI examination and $T_{max}$ is the time point at $SI_{max}$.

2.3.3 Data analysis

The original images were processed using the Advantage Workstation (ADW 4.6 version, GE, US) and post-processed by Functool workstation. Two observers with 15 years and 20 years of experience in MRI were blinded to the information and individually measured the resulting parameter maps. Disagreement of both radiologists was resolved in consensus. All data were measured for 3 times and the average of 3 times is taken to reduce the bias caused by measurement error. On the IVIM-DWI and DCE-MRI series, an ovoid region of interest (ROI) was placed within the bilateral sacral or iliac bone marrow. The ROI of the joint space was placed at the lower third of the cartilaginous portion of the SIJ[11]. All ROIs were 2-4mm$^2$. Then the bilateral average values were taken. Care was taken to avoid cortex, venous plexus, ligaments, or any imaging-related artifacts (Figure 1, 2, 3).

2.4 Histological assessment

After MR examination, all rats were weighed and each rat was euthanized with 1% pentobarbital (Sigma company) 100mg/kg intraperitoneal injection. When heartbeats had not been detected for five minutes, samples of the SIJ were cut across the midline and removed, fixed in 10% formalin (200ml per sample) for one-two day, acid-decalcified with 10% methanoic acid for one week, embedded in paraffin, cut after dehydration in graded ethanol (75% ethanol 15s, 85% ethanol 10s, 95% ethanol 10s, absolute ethanol 1min, absolute ethanol 1min,) and stained with hematoxylin (8min) and eosin (2min). The pathological changes of the SIJ were observed under microscope (MODEL
2.5 Statistical Analysis

SPSS Statistics version 19.0 was used for statistical analysis. The homogeneity of variances was tested using the Levene’s tests. All the parameter values were compared by one-way ANOVA. The measured parameters were expressed as means ± standard deviation (SD). P values of less than 0.05 were considered as statistically significant.

Results

3.1 Performance of Wistar rats

Over the entire study period, there was no statistically significant difference in appearance (including paw, hair, tail, spine, etc.) among the five male Wistar rats of different weeks.

3.2 Results and statistical analysis of various parameters of the SIJ in normal rats with IVIM-DWI sequence

The values of D (×10^{-4} mm^2/s) in the sacrum and in the iliac bone marrow of normal rats in different age groups decreased with the increase of age, and ANOVA indicated a significant difference in D values among different age groups (P=0.000) (Table 1-2). There was no statistically significant difference among the D, D^* and f values of the joint space in SIJ of different age groups (P>0.05). The normal values of corresponding parameters are shown in Table 3. IVIM-DWI images of the SIJ in normal rats are shown in Fig 1.

Table 1. Test of IVIM parameters among different groups by ANONA in the bone marrow of the sacrum.
Table 2. Test of IVIM parameters among different groups by ANOVA in the bone marrow of the iliac bone

| Group    | D (×10^-4 mm^2/s) | D* (×10^-4 mm^2/s) | f(%)     |
|----------|-------------------|--------------------|----------|
| 8 weeks  | 4.84±0.43         | 28.99±4.80         | 24.45±4.84 |
| 13 weeks | 4.63±0.40         | 27.37±3.87         | 26.83±3.97 |
| 18 weeks | 4.76±0.61         | 27.76±3.18         | 25.54±4.87 |
| 23 weeks | 4.34±0.63         | 26.50±2.89         | 24.81±2.89 |
| 28 weeks | 3.89±0.34         | 26.88±4.19         | 24.63±3.26 |
| 33 weeks | 3.67±0.37         | 25.66±2.80         | 24.36±3.54 |
| F        | 26.673            | 1.581              | 1.152    |
| P        | 0.000             | 0.157              | 0.336    |

Table 3. Normal values of IVIM-DWI parameters in the sacral and iliac bone marrow and the joint space in the SIJ of normal rats
### 3.3 Results and statistical analysis of various parameters of SIJ in normal rats with DCE-MRI sequence

The values of Fenh(%) and Senh(%/s) in the sacral and iliac bone marrow and the joint space in the SIJ were not statistically significantly different with the increase of the age \( (P>0.05) \). The normal values of corresponding parameters are shown in Table 4. Through the observation and analysis of the dynamic enhancement curve, the sacral bone marrow showed a type I curve (rapid rise and slow drop, Fig 2); the iliac bone marrow and the joint space in the SIJ of normal rats showed a type II curve (platform, Fig 3).

#### Table 4. Normal values of DCE-MRI parameters in the sacral and iliac bone marrow and the joint space in the SIJ of normal rats

|                          | sacral bone marrow | iliac bone marrow | the joint space |
|--------------------------|--------------------|-------------------|-----------------|
| \( D(\times10^{-4}\text{mm}^2/\text{s}) \) | -                  | -                 | 6.28±0.28       |
| \( D^*(\times10^{-4}\text{mm}^2/\text{s}) \) | 27.31±3.90         | 29.62±5.14        | 61.85±14.93     |
| \( f(\%) \)              | 25.21±3.79         | 26.98±3.38        | 19.85±3.82      |

### 3.4 Pathological results

In six groups of rats with different weeks of age, the SIJ surface was smooth and clear, the cartilage cells were intact, the synovium was normal, and no thickening or pannus formation was found (Figure 4).

Discussion
The SIJ participates in most of the body movements, which is essential for maintaining human coordination. Due to the complex anatomical structure and deep position of the SIJ, it is difficult to conduct general examinations. Therefore, the diagnostic evaluation of the SIJ is mainly based upon medical imaging modalities. Ordinary X-ray examination can only show the general shape of the SIJ, and the value of X-ray in clinical diagnosis, treatment and follow-up is insufficient. CT can clearly show the osteoarticular surfaces and subarticular trabecular bone, but the display of articular cartilage and bone marrow changes is insufficient[12]. MRI can show the bone marrow cavity, the articular cartilage and the surrounding soft tissue very well. However, it is difficult to find early lesions by traditional MRI examination, and the extent of the lesion cannot be quantified[13,14]. The application of new MRI scanning technologies like IVIM-DWI and DCE-MRI can gradually compensate for these shortcomings.

Due to the difficult access for pathology, animal models have special value and would be of great benefit for investigative purposes. Our study lays a foundation for the pathogenesis of ankylosing spondylitis in rats and provides opportunities to investigate their pathology in relationship to human disease.

As we all know, the apparent diffusion coefficient (ADC) is the most commonly used metric in the mono-exponential model (MEM) of DWI, which does not consider the influence of the microcirculation of blood in capillaries, thus leading to an inaccurate description of the diffusion process.[15]. In fact, there are two main aspects that affect the measured diffusion signals in living tissues, On the one hand, the motion of water molecules. On the other hand, the perfusion of the tissue microvasculature. In 1986, Le Bihan et al. described a new imaging technique named IVIM using multi b-value DWI with a bi-exponential curve fitting [10]. This technique is sensitive not only to molecular diffusion in tissues, but also to random flow of blood in capillaries, providing related analytical parameters represented by diffusion-related parameters D (pure molecular diffusion) and perfusion-related parameters including D* (pseudo-diffusion coefficient) and f (perfusion fraction). Semi-quantitative DCE-MRI using a generalised kinetic model allows for estimation of tissue perfusion and capillary permeability, which necessarily involves not only exchange dynamics between extra-vascular/extra-
cellular space, but also some other factors, such as the pattern of blood delivery, blood vessel density, vascular permeability, and distribution of contrast agent in lesions[16]. So far, Many studies have demonstrated the efficiency of IVIM-DWI and DCE-MRI parameters in prostate cancer[17], breast cancer[18] and liver fibrosis[19]. However, there are few reports about the SIJ. The results show that the D-values of the sacral and iliac bone marrow decreased with the increase of age. This difference was statistically significant ($P=0.000$). On the contrary, D* and f did not have statistically significant different results. The reason for these results may be that the main components in the bone marrow cavity of the normal sacrum and ilium are bone trabeculae and bone marrow. The main components of bone trabeculae are minerals such as calcium salts. Here, almost no water molecules can be found, so little to no effect on MR images is expected. The bone marrow is divided into two types: yellow and red bone marrow. When the human body is born, the bone marrow cavity is filled with red bone marrow. As the age increases, part of the red bone marrow is replaced by yellow bone marrow. The red bone marrow contains different stages of red blood cells, white blood cells and platelets. However, the main component of yellow bone marrow is fat, so the ratio of water molecules contained in the two different types of bone marrow is different as the cellular component of red bone marrow contains a lot of water and the fatty yellow bone marrow doesn't. Water content and different states of water molecules can affect the image characteristics and corresponding parameters[20]. Rats aged 8 weeks to 32 weeks old correspond to human beings aged 12 to 20 years old, and the bone marrow in the SIJ has begun to transform from red bone marrow to yellow bone marrow. At the same time, the content of free water molecules in the bone marrow decreases gradually. Under the combined effect of both, the D value reflecting the true water molecule diffusion also decreases with the increase of the age. However, there was no lesion, no inflammatory reaction or necrosis. Therefore, there was no significant difference in D* and f values with the increase of age.

In this study, the initial signal intensity ($S_{I0}$), the maximum signal intensity ($S_{I_{max}}$) and the peak time ($T_{max}$) were obtained by using the semi-quantitative analysis. The enhancement slope and factor were obtained by the signal time-intensity curve (TIC) of the region of interest of normal rats. There
were 180 TIC curves of the SIJ bone marrow region (sacral, iliac) and of the synovial area of the joint space. On the sacral side, a type I curve is seen (with a rapid rise and slow drop). In the synovial area and at the iliac side, a type II curve is seen (with a rapid rise and a platform). There were no significant differences in Fenh (%) and Senh (%/s) between the sacral and iliac bone marrow with an increase in age.

In six groups of rats with different weeks of age, the SIJ surface was smooth and clear, the cartilage cells were intact, the synovium was normal, and no thickening or pannus formation was found. This study was funded by grants from the Natural Science of Shandong Provincial Foundation, China (No.ZR2017MH105) and Academic Promotion Programme of Shandong First Medical University (No.2019QL017).

Conclusions
In conclusion, combined with pathological results, the range of IVIM-DWI and DCE-MRI parameters of sacrum, ilium bone marrow and synovial area of joint space in normal rats were obtained, which laid a foundation our future research. It also provides a basis for further clinical study of SIJ lesions.

Abbreviations
SIJ—Sacro-Iliac Joint
IVIM-DWI—Intravoxel Incoherent Imaging Diffusion Weighted Imaging
DCE-MRI—Dynamic Contrast Enhanced Magnetic resonance imaging
ROI—Region of Interest
TIC—Time-Intensity Curve
ANOVA—Analysis of Variance

Declarations
Ethics Approval and Consent to Participate
The study was approved by the Institutional Animal Care and Use Committee and was performed in accordance with the National Institutes of Health guidelines for the use of laboratory animals.

Consent to publish
Not applicable.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors' Contributions**

JQ, QQY, XBG, JZZ, ZLY, XQL and CQL designed the study. JQ and QQY performed the data analysis and carried out the histological assessment, XBG contributed to the data interpretation. ZLY, XQL and CQL supervised the project. JQ wrote the manuscript and QQY, XBG, JZZ, ZLY, XQL and CQL made contributions to its final form. All authors have read and approved the manuscript in this form.

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Figures
Figure 1

IVIM-DWI images of the SIJ in normal rats, (A-D) Axial T2-weighted FS image, D, D*, f
The TIC of the bilateral sacral bone marrow of the 28-week rat is a type I curve (rapid rise/steep slope and slow drop type).

The TIC of the bilateral iliac bone marrow in the 23-week rat is a type II curve (platform/plateau type).
Figure 4

Clearly showing the synovium (black arrow), chondrocytes (white arrow), sacroiliac surface (blue arrow). The magnification is 200 ×.

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