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Original Article

Quantitative magnetic resonance imaging for diagnosis of intervertebral disc degeneration of the cervico-thoracic junction: a pilot study

Zhihua Han[1,2*], Liang Gao[3*], Qinglei Shi[4], Lei Chen[1], Chun Chen[1,5]

[1]Department of Orthopaedic Surgery, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, PR China; [2]Frankfurt Initiative for Regenerative Medicine, JW Goethe-University, Frankfurt am Main 60528, Germany; [3]Center of Experimental Orthopaedics, Saarland University, Homburg/Saar 66421, Germany; [4]Siemens Ltd., China Healthcare Sector MR Business Group, Beijing 100102, PR China; [5]Engineering Research Center of Clinical Functional Materials and Diagnosis & Treatment Devices of Zhejiang Province, Wenzhou Institute of Biomaterials and Engineering, Wenzhou 325001, PR China. [*]Equal contributors and co-first authors.

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Abstract: This study aimed to appraise two quantitative magnetic resonance imaging techniques, T2* imaging and diffusion-weighted imaging (DWI), for the diagnosis of the intervertebral disc degeneration of the cervico-thoracic junction. Influence of specific factors and diagnostic accuracy of both techniques were particularly explored. Sixty-one volunteers with neck and upper back pain were recruited and evaluated with both T2* imaging and DWI. The Pfirrmann grade, T2* relaxation time and apparent diffusion coefficient (ADC) value of each disc between C7 and T3 were recorded. Stratified analyses were performed for different anatomic levels, genders, age ranges and Pfirrmann grades. The diagnostic accuracy of both techniques was investigated using the receiver operating characteristic (ROC) curves. No statistically significant difference of either T2* relaxation time or ADC value was detected between males and females. Both parameters decreased with the increasing age and Pfirrmann grade. The ROC curves showed the higher sensitivity and specificity for T2* imaging than DWI to quantitatively identify the disc degeneration. Particularly, T2* imaging allowed for a quantitative distinguishing the normal, mild and moderate disc degeneration from the severe degeneration, which was unable to accomplish with DWI. In conclusion, we demonstrated that T2* imaging possess a better accuracy than DWI to quantitatively diagnose the intervertebral disc degeneration at the cervico-thoracic junction.

Keywords: Quantitative MRI, cervico-thoracic junction, intervertebral disc degeneration

Introduction

Intervertebral disc degeneration of the cervical spine is the primary cause of the neck and upper back pain [1]. Recently, the intervertebral disc degeneration occurring between the 7th cervical (C7) and the 3rd thoracic (T3) vertebrae, termed the cervico-thoracic junction, was reported to yield the similar clinical symptoms as the cervical disc degeneration [2]. Specifically, the disc herniations at C7-T1 contribute to estimated 4% to 8% of all cervical disc herniations [3, 4]. Disc herniations at T1-T2 [5-7] and T2-T3 [8-10] were also highlighted. However, in spite of such clinical relevance of the cervico-thoracic junction, few studies had ever shed special light on the radiographic diagnosis of the intervertebral disc degeneration at this crucial region.

Clinical magnetic resonance imaging (MRI) is widely applied to evaluate the intervertebral disc degeneration. Traditionally, the T2-weighted imaging is utilized for the morphological evaluation with the Pfirrmann grading system, reporting a categorical grade ranging from I (normal) to V (severe degeneration) [11]. However, such a subjective grading system is soly based on the qualitative visual inspection of the images and only provides a semi-quantitative assessment with an insufficient accuracy [2, 12-14]. Moreover, cell-based therapies have recently

emerged as alternatives of the traditional approaches to treat the intervertebral disc diseases [15-25], which were proved to be more effective in the mild (grade II) and moderate (grade III) degenerate discs rather than in the severe ones (grade IV-V) [24]. For these costly and grade-dependently effective cell-based therapies, selecting the appropriate candidates is not only clinically critical but also economically meaningful. Also, the outcomes of these cell-based therapies can be assessed by monitoring the biochemical changes within the degenerative discs, which are unable to perform with by traditional MRI. Therefore, more sophisticated MRI techniques with enhanced accuracy are clinically warranted to quantitatively identify the intervertebral disc degeneration and to supervise the outcomes of these cell-based therapies.

Newly emerging quantitative MRI techniques, such as T2 star (T2*) imaging and the diffusion-weighted imaging (DWI), have recently received considerable clinical attention [11-13, 26-31]. T2* imaging is a multi-echo gradient-echo technique and calculates the T2* relaxation time, which has been demonstrated as a reliable indicator of the degeneration of both articular cartilage [26, 27] and intervertebral disc [11, 28, 29]. For the degenerate intervertebral disc, a shorter T2* relaxation time specifies the decreased glycosaminoglycan content and the altered biomechanical characteristics within the disc [11, 29, 30], displaying an inverse correlation between the T2* relaxation time and the Pfirrmann grade [31, 32]. In contrast, DWI measures the diffusion of water within the discs *in vivo* [33] and generates the apparent diffusion coefficient (ADC) value to estimate the free diffusion of the unbound water [34]. The decrease of the glycosaminoglycan or water content in the nucleus pulposus yields a direct decline of the ADC value [35], also demonstrating a negative correlation between the ADC value and the Pfirrmann grade [34]. Despite a considerable number of literature have been published on both T2* imaging and DWI for the evaluation of the disc degeneration, to the best of our knowledge, no previous study has compared these two techniques for the quantitative evaluating the disc degeneration at the cervico-thoracic junction.

This pilot study aimed to evaluate T2* imaging and DWI for the quantitative diagnosis of the intervertebral disc degeneration at the cervico-thoracic junction. Influences of specific factors (anatomical level, gender, age, and degenerative grade) and the diagnostic accuracy of both techniques were particularly investigated.

Materials and methods

Patient selection

This study was approved by the institutional review board of the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, PR China. Each participant was given fully informed consent of the purposes and the potential risks of this study. The participants were recruited following the criteria previously reported: (1) the presence of the symptoms of neck and upper back pain, including the neck or arm weakness, numbness, or tingling, and (2) such symptoms severe enough for the patient to seek the medical treatments [2]. Subjects were excluded if they had: (1) orthopedic implants, pacemakers, aneurysm clips or other ferromagnetic foreign bodies, which are contraindicated by the MRI unit, (2) history of spinal fracture or back surgery, and (3) major systemic diseases and serious illnesses (e.g. osteoporosis, diabetes mellitus, and tumors).

Magnetic resonance imaging, data processing, and image analysis

Standard MRI of the cervico-thoracic junction was obtained for all included participant with a 3.0 Tesla MR scanner (Magnetom Skyra, Siemens Healthcare, Erlangen, Germany) at a fixed time in the afternoon to minimize the diurnal variation on the T2* relaxation time of the disc. Four MRI sequences were applied to each participant including T1-weighted imaging (T1-WI), T2-weighted imaging (T2-WI), T2* imaging and DWI (Table S1). Firstly, the sagittal T1-WI and T2-WI fast spin echo were applied for the morphological images of the intervertebral discs, which were used for the Pfirrmann grading. Next, the sagittal T2* imaging and DWI were performed to investigate the T2* relaxation time and the ADC value of the discs. Particularly, morphological grading of the intervertebral disc degeneration was independently performed by two experienced radiologists in consensus, each acquired more than 10-year experience in the musculoskeletal radiology. Degeneration of each intervertebral disc was

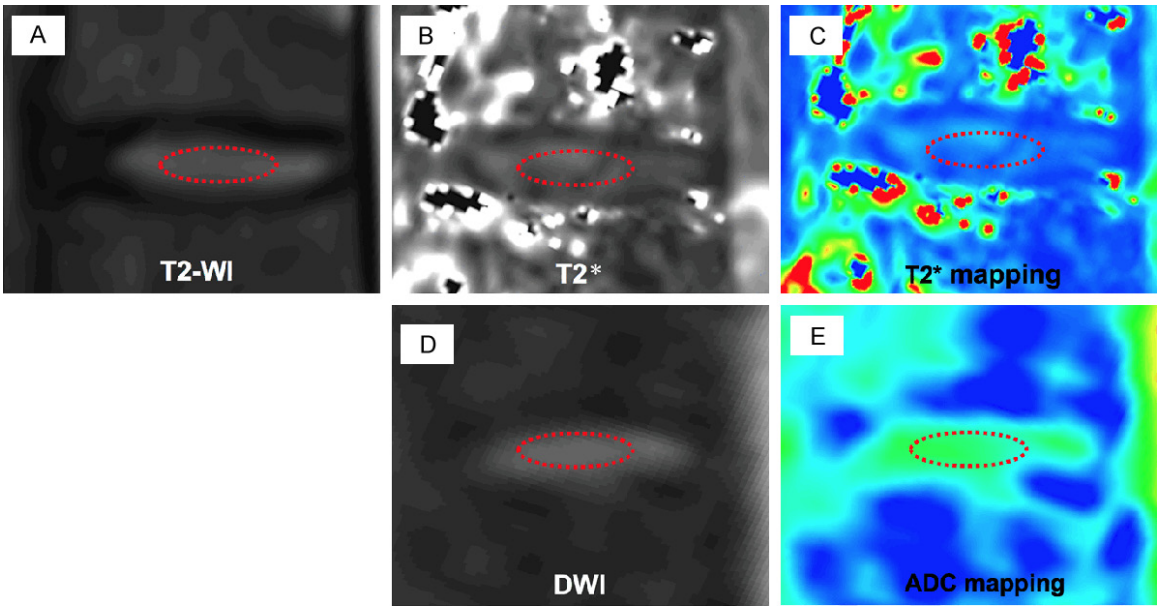

Figure 1. Representative MRI processing and region of interest (ROI) selection. (A) T2-WI image of T2-T3 was obtained from a 43-year old male patient with a red ellipse ROI placed in the middle of the disc to define the nucleus pulposus. The present disc was classified as grade III according to the Pfirrmann grading system with inhomogeneous morphological structure, slightly decreased disc-height, and intermediate signal intensity, which was unclear to distinct between the nucleus and the anulus. The ROI was then copied from the T2-WI image and pasted onto the identical position of the disc on T2* image (B), T2* colored mapping (C), DWI image (D), and ADC colored mapping (E).

Table 1. Number of degenerated intervertebral discs of the cervico-thoracic junction according to the Pfirrmann grade and the anatomic level

Pfirrmann grade	Anatomical level			Total
	C7-T1	T1-T2	T2-T3	
I	21	11	14	46
II	16	8	8	32
III	10	5	10	25
IV	8	5	0	13
V	6	3	0	9
Total	61	32	32	125

graded using the Pfirrmann grading system featured by the change of intensity of the nucleus pulposus, the height of the intervertebral disc, and the distinction between the nucleus and the annulus [34].

Data processing and image analysis of T2* and DWI images were performed on a dedicated workstation (Syngo Multimodality Workplace, Erlangen, Germany) by another radiologist with more than 5-year experience in the spine MR imaging. The T2* relaxation time and the ADC value of the intervertebral discs were achieved following a meticulous setting the regions of interest (ROI) on the nucleus pulposus. To minimize the error in identifying anatomic nucleus pulposus tissue, a free-hand tool was used for manually drawing of the ROIs on the inner portion of each disc as previously described [2, 31]. The ROIs were carefully matched to the nucleus pulposus shape shown on the middle slice of all sagittal T2-WI images (**Figure 1A**). These ROIs on T2-WI images were then copied to T2* and DWI images at the same anatomic position (**Figure 1B-E**).

Comparison of T2 relaxation time and ADC value at different anatomic level, gender, age range and Pfirrmann grade*

Results of both the T2* relaxation time and the ADC value were classified based on the anatomical level, gender, age, and degeneration grade. The age of all participants was categorized into groups with 5 successive age ranges (20-29, 30-39, 40-49, 50-59, and ≥ 60 years old) and analyzed separately. Furthermore, the correlations between the Pfirrmann grade and both the T2* relaxation time and the ADC value were individually evaluated.

Table 2. Comparison of the T2* relaxation time and the ADC value between female and male patients at different anatomical levels of the cervico-thoracic junction

Anatomical level	T2* relaxation time (ms)		*P* value	ADC value (× 10^{-3} mm^2/s)		*P* value
	Female	Male		Female	Male	
C7-T1	22.06 ± 8.12	25.07 ± 7.25	0.13	1.92 ± 0.56	2.22 ± 0.39	0.02
T1-T2	24.26 ± 8.26	24.12 ± 7.45	0.96	1.97 ± 0.33	2.09 ± 0.62	0.57
T2-T3	25.45 ± 5.16	26.39 ± 5.09	0.61	2.00 ± 0.31	2.28 ± 0.39	0.06

ADC: apparent diffusion coefficient; ms: millisecond; mm: millimeter; s: second.

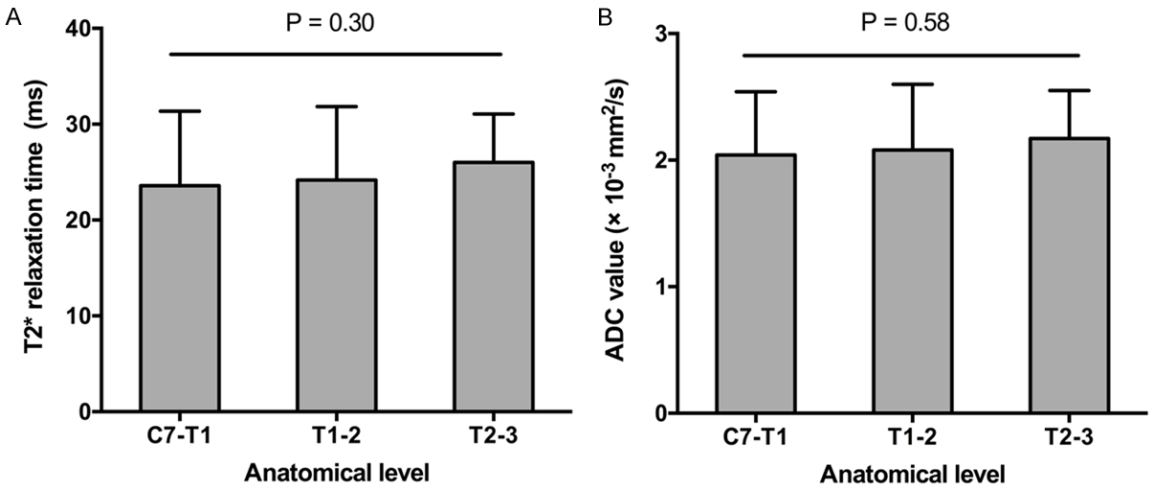

Figure 2. Comparison of T2* relaxation time and ADC value at different anatomical levels. A. No significant difference of the T2* relaxation time at different anatomical levels was detected (P = 0.30). The T2* relaxation time at C7-T1, T1-T2, and T2-T3 was 23.59 ± 7.77 ms, 24.18 ± 7.66 ms and 26.01 ± 5.06 ms, respectively. B. The ADC value slightly increased from C7-T1 to T2-T3 without significant differences (P = 0.58). The ADC value at C7-T1, T1-T2, and T2-T3 was 2.08 × 10^{-3} ± 0.50 × 10^{-3} mm^2/s, 2.04 × 10^{-3} ± 0.52 × 10^{-3} mm^2/s and 2.17 × 10^{-3} ± 0.38 × 10^{-3} mm^2/s, respectively.

Diagnostic accuracy to determine the grade of intervertebral disc degeneration

Receiver operating characteristic (ROC) curves were plotted to test the sensitivity and specificity of both the T2* relaxation time and the ADC value to assess the intervertebral disc degeneration at each Pfirrmann grade. The cut-off values of each ROC curve were determined by choosing the points indicating the maximum "sensitivity and specificity" values as described elsewhere [36]. Also, the area under the curve (AUC) values were calculated for each curve.

Statistical analysis

Values are expressed as mean ± standard deviation (SD) for continuous variables. All statistical analyses were performed using SPSS 24.0 (SPSS, Chicago, Illinois, USA). One-way analysis of variance (ANOVA) and Student *t*-test were used to investigate the differences of the T2* relaxation time and ADC value of different anatomic levels, genders, age ranges and Pfirrmann grades. Spearman rank correlation was performed to assess the correlation of T2* relaxation time or ADC values with either age or Pfirrmann grade, respectively. Statistical significance was set at P < 0.05.

Results

Demographic characteristics of patients

A total of 61 symptomatic participants were enrolled (30 females, 31 males) after screening with the above-mentioned criteria. The age of the enrolled participants ranged from 24 to 76, with 18 participants between 20-29 years old, 9 between 30-39, 13 between 40-49, 10 between 50-59, and 11 older than 60. One hundred and twenty-five intervertebral discs at

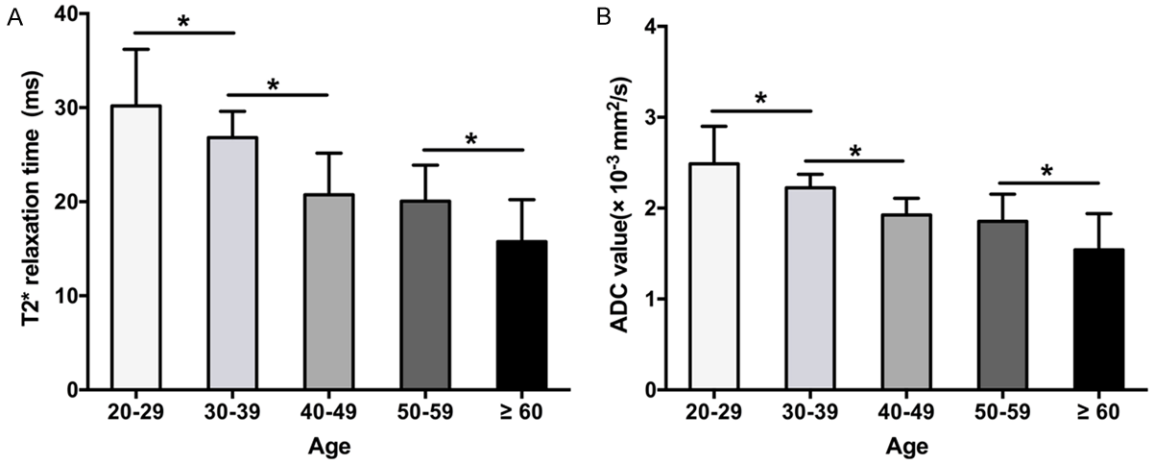

Figure 3. Comparison of T2* relaxation time and ADC value at different age ranges. A. The T2* relaxation time decreased evidently with the increased age, from 30.19 ± 6.01 ms at the age of 20-29 to 15.75 ± 4.47 ms at the age over 60, and significant differences were detected between each two adjacent age ranges, except between 40-49 and 50-59. B. The ADC value significantly declined with the increasing age by 0.95 × 10^{-3} mm^2/s from the age of 20-29 (2.49 × 10^{-3} ± 0.41 × 10^{-3} mm^2/s) to the age over 60 (1.54 × 10^{-3} ± 0.40 × 10^{-3} mm^2/s) and similar significant differences were seen between each two neighboring age ranges, except between 40-49 and 50-59. *P < 0.05.

the cervico-thoracic junction without the magnetic susceptibility artifacts were used for the further analysis. Specifically, 26 discs (43%) at C7-T1 were graded as the mild or moderate degeneration (grade II-III) and 14 discs (23%) were graded as severe degeneration (grade IV-V) (**Table 1**). At T1-T2, 13 discs (41%) were rated as the mild or moderate degeneration (grade II-III) and 8 discs (25%) were rated as the severe degeneration (grade IV-V). At T2-T3, 18 discs (56%) were sorted as the mild or moderate degeneration (grade II-III) and no disc was sorted as the severe degeneration (grade IV-V).

Comparison of T2 relaxation time and ADC value at different anatomic level*

The T2* relaxation time and the ADC value were obtained and arranged according to the anatomical level and gender (**Table 2**). Regarding the anatomic level, the T2* relaxation time at C7-T1, T1-T2, and T2-T3 was 23.59 ± 7.77 ms, 24.18 ± 7.66 ms and 26.01 ± 5.06 ms, respectively. The ADC value at C7-T1, T1-T2, and T2-T3 was 2.08 × 10^{-3} ± 0.50 × 10^{-3} mm^2/s, 2.04 × 10^{-3} ± 0.52 × 10^{-3} mm^2/s and 2.17 × 10^{-3} ± 0.38 × 10^{-3} mm^2/s, respectively. No significant diffference was detected when comparing either the T2* relaxation time or the ADC value among the different anatomical levels (P = 0.30 and P = 0.58, respectively; **Figure 2**).

Comparison of T2 relaxation time and ADC value at different gender*

No statistically significant difference existed comparing either the T2* relaxation time or the ADC value between males and females at each anatomical level except at C7-T1 (**Table 2**). As to the T2* relaxation time, the males generally showed the higher mean values than the females without reaching statistical significance at C7-T1 (25.07 ± 7.25 ms *versus* 22.06 ± 8.12 ms, P > 0.05) and T2-T3 (26.39 ± 5.09 ms *versus* 25.45 ± 5.16 ms, P > 0.05), except at T1-T2 (24.12 ± 7.45 ms *versus* 24.26 ± 8.26 ms, P > 0.05) (**Table 2**). Similarly, regarding the ADC value, the male patients showed the higher mean ADC value at all anatomical levels, however, the statistical significance was only yielded at the level of C7-T1 (1.92 ± 0.56 × 10^{-3} mm^2/s *versus* 2.23 ± 0.39 × 10^{-3} mm^2/s, P = 0.02).

Comparison T2 relaxation time and ADC value between different age range*

Both the T2* relaxation time and the ADC value decreased with the increasing age. The T2* relaxation time declined from 30.19 ± 6.01 ms at the age of 20-29 to 15.75 ± 4.47 ms at the age over 60 (Table S2). Similarly, the mean ADC value decreased by 0.95 × 10^{-3} mm^2/s from the age of 20-29 (2.49 × 10^{-3} ± 0.41 × 10^{-3} mm^2/s)

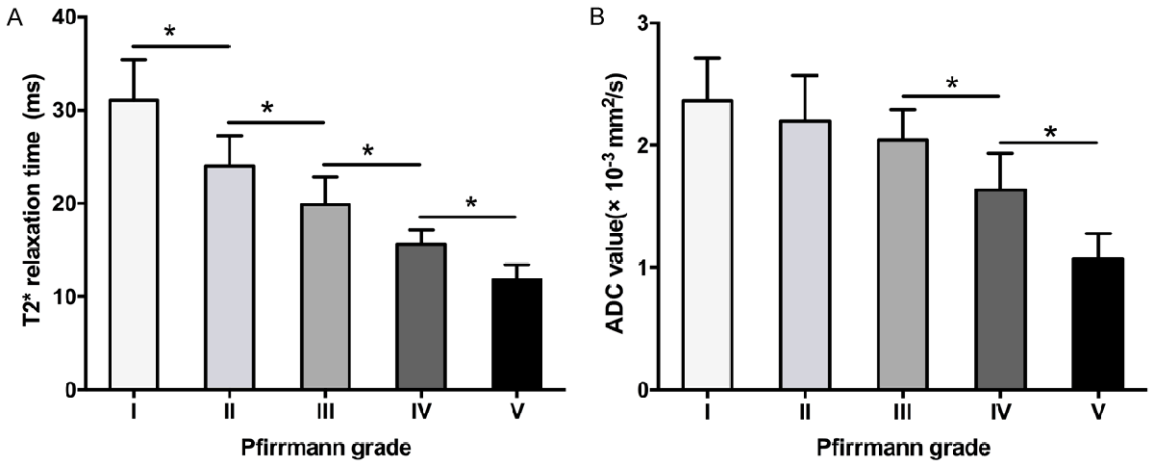

Figure 4. Comparison of T2* relaxation time and ADC value at different Pfirrmann grade. A. The T2* relaxation time significantly decreased from grade I (31.03 ± 4.38 ms) to V (11.79 ± 1.60 ms) with statistical significance existed in the comparison of each two adjacent Pfirrmann grades (all $P < 0.05$). B. The ADC value generally decreased with the advance of the Pfirrmann grade, from $2.36 \times 10^{-3} \pm 0.35 \times 10^{-3}$ mm^2/s at grade I to $1.07 \times 10^{-3} \pm 0.21 \times 10^{-3}$ mm^2/s at grade V. Significant differences of the ADC value were only observed among grade III, IV, V. *$P < 0.05$.

to the age over 60 ($1.54 \times 10^{-3} \pm 0.40 \times 10^{-3}$ mm^2/s) (Table S2). The multiple comparisons of ANOVA analysis demonstrated statistical significances for both T2* relaxation time and ADC value between every two adjacent age ranges (all $P < 0.05$), except between 40-49 and 50-59 (**Figure 3**; Table S2). The mean T2* relaxation time was 20.74 ± 4.41 ms at the age 40-49 and slightly decreased to 20.06 ± 3.83 ms at the age 50-59 without reaching statistical significance ($P > 0.05$), while the comparison of the ADC value between the age 40-49 and 50-59 also showed no statistically significant difference ($P > 0.05$).

Comparison T2 relaxation time and ADC value between different degeneration grade*

Both the T2* relaxation time and the ADC value tended to decrease with the advance of the Pfirrmann grade. A significant negative correlation was observed between either the T2* relaxation time or the ADC value and the Pfirrmann grade. Particularly, the T2* relaxation time decreased from 31.03 ± 4.38 ms at grade I to 11.79 ± 1.60 ms at grade V with statistically significant differences between each two adjacent grades (all $P < 0.05$) (**Figure 4**; Table S3). Furthermore, a significant negative correlation between the T2* relaxation time and the degeneration grade was detected ($r = -0.89$, $P < 0.001$). Likewise, the ADC value showed a decline from $2.36 \times 10^{-3} \pm 0.35 \times 10^{-3}$ mm^2/s at grade I to $1.07 \times 10^{-3} \pm 0.21 \times 10^{-3}$ mm^2/s at grade V. The ADC value was also negatively correlated to the Pfirrmann grade ($r = -0.62$, $P < 0.001$) (**Figure 4**; Table S3). Interestingly, although significances existed when comparing the ADC value of either grade IV or grade V with that of other grades (all $P < 0.05$), multi-comparison tests detected no significant difference of the ADC value among grade I, II and III (all $P > 0.05$).

Diagnostic accuracy of T2 imaging and DWI*

The ROC curves showed T2* imaging obtained the better sensitive and specificity than DWI to quantitatively diagnose the disc degeneration of the cervico-thoracic junction. Principally, the T2* relaxation time allowed for a quantitative distinguishing the normal (grade I), mild (grade II) and moderate (grade III) disc degeneration from the severe degeneration (grade IV-V), which was unable to accomplish with the ADC value (**Figure 5**; Table S4). The T2* cut-off values between each of two advancement Pfirrmann grades were selected to be 26.5, 21.7, 18.0 and 13.9 ms, respectively (Table S4). The AUC values of all ROC curves of T2* imaging ranged from 0.84 to 0.97 (**Figure 5**; Table S4), and all T2* relaxation time cut-off values were yielded with their sensitivities from 0.78 to 0.92 and specificities from 0.76 to 1.00. Regarding the ADC value, The cut-off values between each of two consecutive Pfirrmann

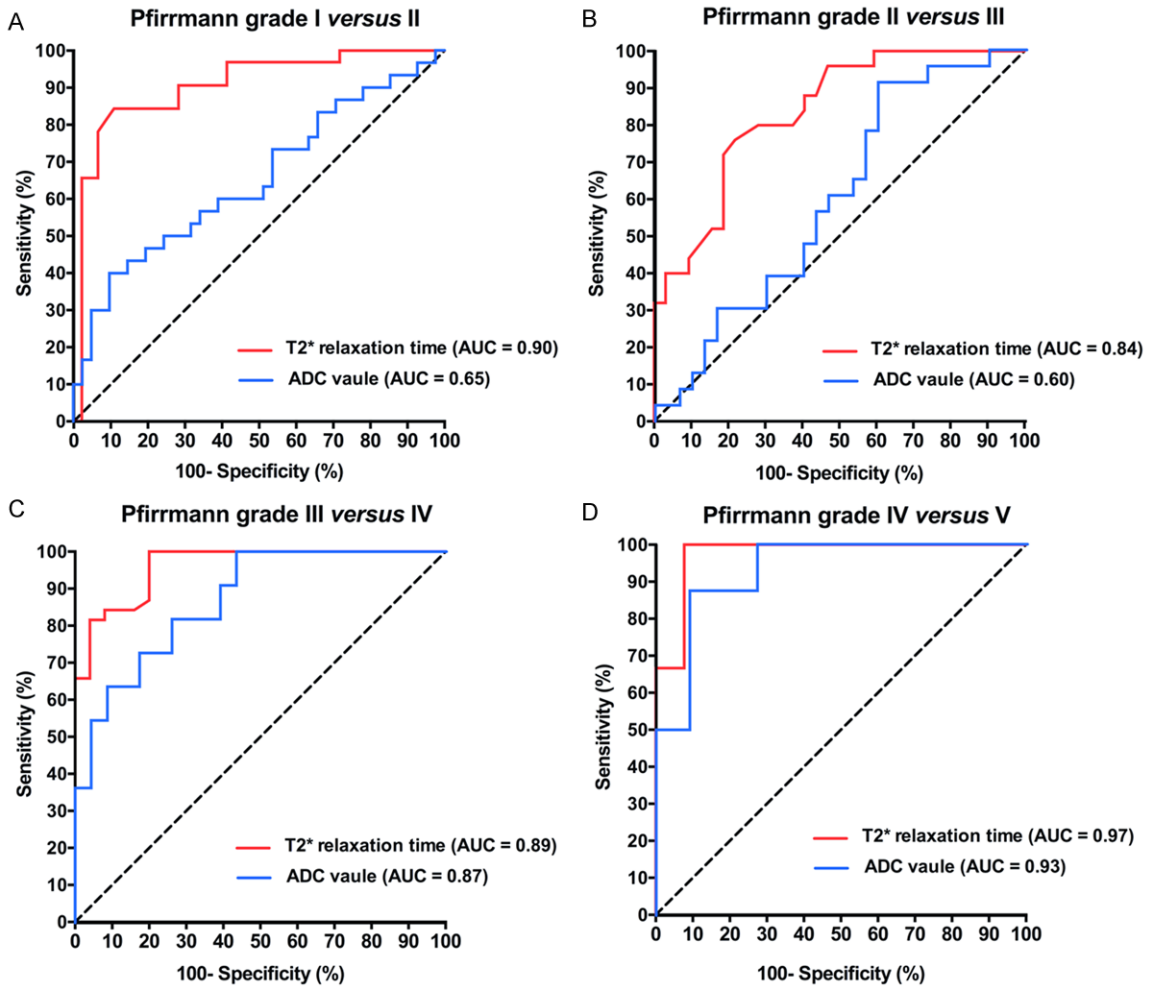

Figure 5. Receiver operating characteristic (ROC) curves to compare the diagnostic accuracy of T2* imaging and DWI for the discrimination between each two consecutive Pfirrmann grades. The T2* cut-off values between each of two advancement Pfirrmann grades were selected to be 26.5, 21.7, 18.0 and 13.9 ms, while the cut-off ADC values between each of two consecutive Pfirrmann grades were chosen to be 2.30×10^{-3}, 2.28×10^{-3}, 2.02×10^{-3} and 1.43×10^{-3} mm^2/s, respectively. A. The ROC curves of T2* relaxation time and ADC value of the grade I *versus* II showed that the area under curve (AUC) of T2* relaxation time (0.90) was evidently larger than that of ADC value (0.65). B. The ROC curves of grade II *versus* III. Similarly, the curve of T2* relaxation time showed a larger AUC than the curve of ADC value (0.84 and 0.6; respectively). C. The ROC curves of grade III *versus* IV. The AUC of the curves of T2* relaxation time and ADC value were 0.89 and 0.87. D. The ROC curves of grade IV *versus* V. The AUC of the curve of the T2* relaxation time (0.97) was approximate to that of the curve of the ADC value (0.93).

grades were chosen to be 2.30×10^{-3}, 2.28×10^{-3}, 2.02×10^{-3} and 1.43×10^{-3} mm^2/s, respectively (Table S4). The AUC values of all ROC curves of the ADC value ranged from 0.65 to 0.93 (**Figure 5**; Table S4), and all ADC cut-off values were yielded with their sensitivities from 0.49 to 0.73 and specificities from 0.63 to 1.00. Generally, the T2* relaxation time obtained higher AUC values of the ROC curves than the ADC value, especially for differentiating grade I and II (0.90 *versus* 0.65), grade II and III (0.84 *versus* 0.60) (Table S4). Moreover, the sensitivity and specificity of the T2* relaxation time were also higher than that of the ADC value at distinguishing each two neighbor Pfirrmann grades. Of note, the values of sensitivity and specificity of the T2* relaxation time at grade I *versus* II were 0.91 and 0.81, while those of the ADC value were 0.49 and 0.63, respectively (Table S4).

Discussion

The most important finding of this study was that T2* imaging possessed a better accuracy than DWI to quantitatively diagnose the inter-

vertebral disc degeneration at the cervico-thoracic junction, especially allowing for identifying the normal, mild and moderate degenerate discs. Also, we proved the negative correlations between the degenerative grade and either the T2* relaxation time or the ADC value of the intervertebral disc at this region.

The exact definition of the cervico-thoracic junction is still under debate and few previous studies have ever focused on the disc degeneration of this region. Miscusi *et al*. proposed the cervico-thoracic junction as the region from the C7 to T4 and their attachments [37], however, other researchers considered the cervico-thoracic junction as the C7 and T1 vertebrae, the disc between C7-T1, and the attachments of the paraspinal soft tissue [1, 3]. In the present study, we defined the cervico-thoracic junction as C7-T1, T1-T2 and T2-T3 and the attachments of the paraspinal soft tissue.

Despite lacking a consensus definition of the cervico-thoracic junction, the intervertebral disc degeneration at the cervico-thoracic junction is as inevitable as other regions of the spine, serving as a clinically critical but often overlooked source of the neck and upper back pain [2, 4, 7]. Previous studies reported that the disc herniation also frequently occurred at T1-T2 [5-7] and T2-T3 [8-10]. Accordingly, our data also revealed the disc degeneration occurring at C7-T3. The mild and moderate degeneration (grade II-III) was generally seen at all anatomical levels (C7-T1: 43%; T1-T2: 41%; T2-T3: 56%) and the severe disc degeneration (grade IV-V) occurred at both C7-T1 (9.8%) and T1-T2 (9.3%) (**Table 2**). Such severe degeneration might result in a future disc herniation or spondylosis.

Our data also revealed that T2* imaging held a better accuracy than DWI to quantitatively diagnose the intervertebral disc degeneration at the cervico-thoracic junction. The ROC curves showed that the T2* relaxation time exhibited better ability than the ADC value with the general larger AUC and the higher "sensitivity and specificity" (**Figure 5**; Table S4). Most importantly, T2* imaging allowed for a definitive distinguishing the normal discs, mild and moderate degenerate discs from the severe degenerate discs, which was unable to accomplish with DWI. In accordance with the ROC curves, the statistical significance was detected in the difference of T2* relaxation time between the normal, mild and moderate degenerate discs (grade I-III) ($P < 0.05$), while such a statistically significant difference was not detected for the ADC value during the same analysis. Similar finding of no significant difference of the ADC value among the grade I-III was reported previously by Nia *et al*. [12] when comparing the T2 relaxation time and the ADC value of the lumbar discs. Clinically speaking, the emerged cell-based therapies for disc degeneration are grade-dependently effective and the outcomes of these treatments could be radiographically monitored by the quantitative MRI techniques. Such a decisively quantitative diagnosis with T2* imaging provides a clinically critical tool to identify the appropriate candidates for these costly cell-based treatments of the intervertebral disc degeneration.

Additionally, the T2* relaxation time showed a stronger inverse correlation with the Pfirrmann grade than the ADC value. In the present study, the correlation between either the T2* relaxation time or the ADC value and the Pfirrmann grade was -0.89 and -0.62, respectively. Such inverse correlations between either T2* relaxation time or ADC value and severity of disc degeneration were also previously reported [12, 13, 28, 31, 34]. Huang *et al*. demonstrated the negative correlations between the T2* relaxation time and the Pfirrmann grade of the degenerated cervical discs ($r = -0.673$) [31]. Niinimaki and colleagues also reported the negative correlations between the ADC value and the Pfirrmann grade of the degenerated lumbar discs [13]. As the first pilot study comparing T2* imaging and DWI in the cervico-thoracic junction, the data of the present study showed the stronger correlation between the Pfirrmann grade and the T2* relaxation time over the ADC value, suggesting the potential advantage of T2* imaging for the quantitatively scaling the intervertebral disc degeneration to complement the clinically applied Pfirrmann grading system.

Interestingly, both quantitative MRI analysis of the caudal discs at the cervico-thoracic junction obtained generally longer T2* relaxation time and higher ADC value. The T2* relaxation time increased by 3.39 ms from C7-T1 to T2-T3, and the ADC value likewise increased from

2.08 ± 0.50 × 10^{-3} mm^2/s at C7-T1 to 2.17 ± 0.38 × 10^{-3} mm^2/s at T2-T3. Such increases might be partially explained by the relative higher range of motion at C7-T1 than that at both T1-T2 and T2-T3 [38]. Also, these data mirror the previous studies that examined the cervical disc degeneration with T2* imaging. Huang and colleagues reported the lowest T2* relaxation time at C5-C6 with the largest range of motion among the entire cervical spine [31]. By contrast in the lumbar disc degeneration, Kealey *et al.* [39] and Niu *et al.* [33] reported the lowest ADC value at L5-S1 among the total lumbar discs mainly due to its greatest biomechanical burden. Therefore, such sophisticated topographic distributions of the movement and stress of the entire spine might underline a further region-specific analysis using the advanced quantitative MRI techniques.

We also found both the T2* relaxation time and the ADC value decreased with the increasing age. The T2* relaxation time decreased by 14.44 ms from the age of 20-29 to the age over 60, the mean ADC value declined from 2.49 × 10^{-3} ± 0.41 × 10^{-3} mm^2/s at the age of 20-29 to 1.54 × 10^{-3} ± 0.40 × 10^{-3} mm^2/s at the age over 60. Similar influences of age on the T2 relaxation time or the ADC value of the degenerated disc were previous reported by many other studies [12, 34, 39-41]. Niu and colleagues demonstrated a more significant inverse correlation between the age and the T2 relaxation time than the ADC value of the lumbar discs in an asymptomatic population [12], also highlighting the possible disadvantage of DWI. Remarkably, all comparisons in the present study of either T2* relaxation time or ADC value at different age ranges reached statistically significant differences except the comparison between the age ranges of 40-49 and 50-59. These data might be explained by the relatively slower degeneration process at the middle age. As reported by Mitchell *et al*. [42], the middle-age individuals could regain more water diffusion after the rehabilitation activities than the young adults and achieved a significant higher increase of the ADC value after the static traction.

The relation between the liability of intervertebral disc degeneration and the gender is complex and controversial. Generally, males are more vulnerable to the disc degeneration for the increased mechanical stress and the occupational factors [1]. Such trend turns to its opposite in the elder population, in which females show a higher prevalence of low back pain after the menopause [43]. The present study detected no significant difference in either T2* relaxation time or ADC value between males and females. At each anatomical level, male patients generally had longer T2* relaxation time and higher ADC value than female patients, however no significant influence of the gender on both parameters was detected (all $P > 0.05$), except the ADC value of discs at C7-T1 ($P = 0.02$). These data were consistent with our previous study of the cervical discs in an asymptomatic population [14], in which the shorter T2 relaxation time in females than males was appreciated without reaching statistical significance. Therefore, further studies, taking the age, gender, body weight, occupation, and other influence factors into account, will need to be undertaken.

This study held several limitations. Firstly, this was a pilot study in a single research center with a relatively small sample size and future multiple center studies with larger sample size are required to reconfirm our findings. Secondly, the radiographic findings of the present study were not confirmed by any histological and biochemical tests of biopsy samples, which also lied outside the scope of the current study. Thirdly, only symptomatic volunteers were included and the comparison with healthy controls is recommended for future investigations.

In conclusion, we demonstrated that T2* imaging was more accurate than the diffusion-weighted MRI to quantitatively diagnose the intervertebral disc degeneration at the cervico-thoracic junction. Particularly, T2* imaging allowed for distinguishing the mild and moderate disc degeneration from the severe degeneration, possibly serving as a clinical crucial tool for the emerging cell-based therapies of the intervertebral disc degeneration.

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Disclosure of conflict of interest

None.

Address correspondence to: Chun Chen and Lei Chen, Department of Orthopaedics, The First Affiliated Hospital of Wenzhou Medical University, Nan Baixiang Road, Shangcai Village, Ouhai District, Wenzhou 325000, PR China. Tel: +86 18858703873; E-mail: chenchunkk@163.com (CC); Tel: +86 13957789595; E-mail: chenlei689595@qq.com (LC)

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Table S1. Parameter settings of the MRI sequences

Parameter (Unit)	Sequence			
	T1-WI (sagittal)	T2-WI (sagittal)	T2* (sagittal)	DWI (sagittal)
Repetition (ms)	550	2300	419	4900
Echo time (ms)	9	117	4.36	75
Field of view (mm)	26 × 26	16 × 16	22 × 22	22 × 22
Matrix	320 × 240	320 × 240	288 × 288	192 × 192
Slice thickness (mm)	4	3	4	3
Inter-slice gap (mm)	0.4	0.3	0.4	0.9
Number of slices	7	10	7	12
Echo trains/slice	4	15	-	-
Band width (KHz)	240	284	260	651
Number of signal-intensity acquisition	2	2	2	-
Examination time (second)	120	172	364	305

KHz: kilohertz; T1-WI: T1-weighted imaging; T2-WI: T2-weighted imaging; DWI: diffusion-weighted imaging; min: minute; ms: millisecond; mm: millimeter.

Table S2. Comparison of T2* relaxation time and ADC value at different age ranges

Age range	Number of discs	T2* value (ms)	ADC value (× 10^{-3} mm^2/s)
20 - 29	46	30.19 ± 6.01$^{‡,¶,\#,\&}$	2.49 ± 0.41$^{‡,¶,\#,\&}$
30 - 39	18	26.82 ± 2.79$^{†,¶,\#,\&}$	2.22 ± 0.15$^{†,¶,\#,\&}$
40 - 49	27	20.74 ± 4.42$^{†,‡,\&}$	1.93 ± 0.18$^{†,‡,\&}$
50 - 59	18	20.06 ± 3.83$^{†,‡,\&}$	1.85 ± 0.30$^{†,‡,\&}$
≥ 60	16	15.75 ± 4.47$^{†,‡,¶,\#}$	1.54 ± 0.40$^{†,‡,¶,\#}$
Total	125	24.36 ± 7.16	2.09 ± 0.48

ADC: apparent diffusion coefficient; ms: millisecond; mm: millimeter; s: second. $^{†}P < 0.05$ compared with discs of age range 20-29. $^{‡}P < 0.05$ compared with discs of age range 30-39; $^{¶}P < 0.05$ compared with discs of age range 40-49; $^{\#}P < 0.05$ compared with discs of age range 50-59; $^{\&}P < 0.05$ compared with discs of age ≥ 60.

Table S3. Comparison of T2* relaxation time and ADC value at different Pfirrmann grade

Pfirrmann grade	T2* relaxation time (ms)	ADC value (× 10^{-3} mm^2/s)
I	31.03 ± 4.38$^{‡,¶,\#,\&}$	2.36 ± 0.35$^{‡,¶,\#,\&}$
II	24.01 ± 3.24$^{†,¶,\#,\&}$	2.20 ± 0.37$^{†,¶,\#,\&}$
III	19.88 ± 2.93$^{†,‡,\#,\&}$	2.05 ± 0.25$^{†,‡,\#,\&}$
IV	15.62 ± 1.57$^{†,‡,¶,\&}$	1.64 ± 0.30$^{†,‡,¶,\&}$
V	11.79 ± 1.60$^{†,‡,¶,\#}$	1.07 ± 0.21$^{†,‡,¶,\#}$
Total	24.36 ± 7.16	2.09 ± 0.47

ADC: apparent diffusion coefficient; ms: millisecond; mm: millimeter; s: second. $^{†}P < 0.05$ compared with discs of Pfirrmann grade I; $^{‡}P < 0.05$ compared with discs of Pfirrmann grade II; $^{¶}P < 0.05$ compared with discs of Pfirrmann grade III; $^{\#}P < 0.05$ compared with discs of Pfirrmann grade IV; $^{\&}P < 0.05$ compared with discs of Pfirrmann grade V.

Table S4. Receiver operating characteristic (ROC) curves to compare the diagnostic accuracy of T2* imaging and DWI for the discrimination between each two consecutive Pfirrmann grades

Pfirrmann grade	T2* relaxation time				ADC value			
	Cut-off value (ms)	AUC	Sensitivity	Specificity	Cut-off value ($\times 10^{-3}$ mm^2/s)	AUC	Sensitivity	Specificity
I *versus* II	26.5	0.90	0.91	0.81	2.30	0.65	0.49	0.63
II *versus* III	21.7	0.84	0.78	0.76	2.28	0.60	0.40	0.92
III *versus* IV	18.0	0.89	0.80	1.00	2.02	0.87	0.57	1.00
IV *versus* V	13.9	0.97	0.92	1.00	1.43	0.93	0.73	1.00

ADC: apparent diffusion coefficient; AUC: area under curve; ms: millisecond; mm: millimeter; s: second.