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Diffusion tensor imaging-based quantitative analysis of the spinal cord in Pembroke Welsh Corgis with degenerative myelopathy

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ABSTRACT. Canine degenerative myelopathy (DM) is a progressive neurodegenerative disease of the spinal cord. The diagnosis is based on the observation of clinical signs, genetic testing, and exclusion of other spinal cord diseases, and a definitive diagnosis of DM can only be confirmed by postmortem histopathological findings. The aim of this study was to investigate the diagnostic ability of diffusion tensor imaging (DTI) for DM. Eight DM-affected Pembroke Welsh Corgis, thirteen dogs with thoracolumbar intervertebral disk herniation (IVDH), and six healthy control dogs were included. All dogs were scanned using a 3.0-T MRI system. Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values were calculated for each intervertebral disk level slice between T8–T9 and L2–L3 intervertebral disk levels, and the entire area of the thoracolumbar spinal cord between T8–T9 and L2–L3 intervertebral disk levels (T8–L3 region). The ADC and FA values of the T8–L3 region were significantly lower in the DM group than in the IVDH group. The ADC values for the T8–L3 region had a moderate negative correlation with clinical duration (r_s= −0.723, P=0.043); however, the FA values of other intervertebral disk levels and T8–L3 region had no correlation with clinical durations. The measurement of DTI indices can be used to quantitatively assess neurodegeneration and may have diagnostic value for DM. In particular, the ADC value of the T8–L3 region may aid in making a non-invasive premortem diagnosis of DM.

KEY WORDS: degenerative myelopathy, diffusion tensor imaging, dog, MRI, thoracolumbar spinal cord

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Canine degenerative myelopathy (DM) is a fatal neurodegenerative spinal cord disorder that develops in several breeds, including German Shepard dogs, Boxers, and Pembroke Welsh Corgis (PWC) [2, 10]. Mutations in the Cu/Zn-superoxide dismutase 1 (SOD1) gene have been identified in DM dogs [3, 39]. Age at the onset of clinical signs is 8 years or older in most cases [39]. The initial clinical signs of DM are upper motor neuron spastic paresis of the pelvic limbs, which gradually progresses to flaccid tetraplegia. Eventually, affected dogs usually die from respiratory impairment 3 years after disease onset if euthanasia is not selected during the course of disease progression [10, 29].

Currently, a definitive diagnosis of DM can only be confirmed by postmortem histopathological findings [2, 16, 23]. The clinical diagnosis of DM is based on the following criteria: confirmation of the progression of clinical signs, identification of reported SOD1 mutations, and exclusion of other progressive spinal cord disorders that clinically mimic DM [8, 39]. However, acquired compressive spinal cord disorders can co-exist with DM, confounding the clinical diagnosis. Therefore, the clinical diagnosis of DM with acquired compressive spinal cord disorders is difficult at the time of MRI examination and can only be distinguished based on the observation of subsequent long-term clinical signs. For an earlier and more accurate premortem diagnosis, novel diagnostic tools that can discriminate DM from acquired compressive spinal cord disorders are needed.

The pathological changes in the DM spinal cord are characterized by axonal degeneration, axonal loss, and demyelination of the white matter in the cervical, thoracic, and lumbar spinal cord in the presence of proliferated fibrous and gemistocytic astrocytes [3, 9, 23, 25]. Although these marked histopathological changes occur at every part of the spinal cord [10], conventional magnetic resonance imaging (MRI) techniques fail to depict DM lesions [28]. Diffusion tensor imaging (DTI), which enables *in vivo* quantification of water movement, provides quantitative information on axonal organization in the spinal cord [35]. Measurement

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of the apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values, which are calculated from diffusion-weighted imaging data, has been utilized for the assessment of many neurodegenerative diseases in humans [24]. In a study of amyotrophic lateral sclerosis (ALS), a known human neurodegenerative disorder, FA measurement in the spinal cord was reported as a promising diagnostic technique [30]. In addition, DTI parameters may have predictive value for the survival time and progression rate of respiratory dysfunction in ALS patients [14, 32]. In dogs, spinal cord injury and spinal cord tumors have been investigated by a DTI sequence [20]. A recent study reported that the FA values of the spinal cord in DM was significantly lower than that in healthy control dogs [15]; however, there is no report of DTI-based quantitative analysis of the spinal cord in DM compared with acquired compressive spinal cord disorders.

The aim of the current study was to investigate the diagnostic ability of DTI for DM. We acquired DTI with a 32-direction motion probing gradient using a 3.0-Tesla MRI system, and measured FA and ADC values for the thoracolumbar spinal cord (TLSC) in DM, intervertebral disk herniation (IVDH), and healthy control dogs. Dogs with IVDH was included in this study because IVDH is the most common cause of hindlimb paralysis that needs to be differentiated from DM. We hypothesized that the FA values in DM dogs are lower than those in SCI and healthy control dogs because of the anisotropic reduction in the DM spinal cord due to axonal loss and demyelination. We also hypothesized that the ADC values in DM dogs are lower than those in SCI and healthy control dogs because of restricted movement of water molecules in the DM spinal cord, which is associated with the high cellularity of proliferated fibrous and gemistocytic astrocytes.

MATERIALS AND METHODS

Animals

This study was conducted as a retrospective cross-sectional study. In order to compare the DTI profile of dogs among DM, IVDH, and healthy control groups, this study included dogs that underwent an MRI sequence at the Animal Medical Center of Gifu University between August 2019 and January 2021. All owners signed an informed consent form (approved by the Animal Medical Center of Gifu University and Use Committee, protocol # 2019-113). In the DM group, dogs were diagnosed with DM according to the following criteria: clinical signs consistent with DM (adult onset, slowly progressive, and non-painful paraparesis progressing to tetraplegia) [9, 10], unremarkable findings on spinal cord imaging with conventional MRI sequences, and genetic testing confirming homozygosity for the *SOD1* c.118G >A missense mutation (A/A) [8]. Dogs with IVDH, which had clinical signs consistent with DM and *SOD1* gene mutation at the time of MRI, were included in the DM group if these dogs had progressive clinical signs consistent with DM for more than a year at the time of data analysis. We also included postmortem imaging data in the DM group if the dogs underwent the DTI sequence within 24 hr after death and necropsy. The owners of the dogs were instructed to store the dogs in a cool condition and place refrigerants over the entire spine in order to minimize postmortem changes until they bring the dogs to us. The disease stage of DM was classified into four clinical stages as previously described [9, 10]. Briefly, clinical stages were characterized as follows: Stage 1, general proprioceptive ataxia and upper motor neuron paraparesis; Stage 2, non-ambulatory paraparesis to paraplegia; Stage 3, lower motor neuron paraplegia to thoracic limb weakness; and Stage 4, lower motor neuron tetraplegia and brain stem signs. All dogs in the IVDH group had thoracolumbar spinal cord compression by herniated intervertebral disks, which were confirmed by MRI (Achieva dStream, Philips, Amsterdam, Netherland). Thoracolumbar IVDH cases were graded into five grades as previously described [1, 33]. Briefly, the clinical grading of thoracolumbar IVDH was as follows: Grade 1, thoracolumbar pain only; Grade 2, ambulatory paraparesis; Grade 3, non-ambulatory paraparesis; Grade 4, paraplegia with positive deep pain sensation; and Grade 5, paraplegia with a loss of deep pain sensation. Clinically normal adult dogs with no abnormal findings on spinal cord imaging with conventional MRI sequences were recruited for the control group. Dogs were diagnosed with IVDH according to the following criteria: identification of interruption of the CSF flow at dorsal and ventral site and/or deformity of the cross-sectional area of the spinal cord, which were caused by spinal cord compression. All dogs in the control group had normal neurological examination findings and no history of neurological disorder. Exclusion criteria of this study for the DM or IVDH group were as follows: dogs with an incomplete diagnosis, intracranial disorders, vertebral/spinal cord tumors, and no intramedullary or intradural extramedullary structural lesions. All dogs of these MRI studies were included in a previous study [27].

MRI sequences

All MRI sequences were acquired using a 3.0-Tesla MRI system (Achieva dStream, Philips, Amsterdam, Netherland) with an 8-channel coil as an RF coil. For MRI procedures, general anesthesia was induced with intravenous propofol (PROPOFOL injection, Fuji Pharma Co., Ltd., Toyama, Japan). All dogs were intubated and maintained with a mixture of isoflurane (Isoflurane, Pfizer Inc., New York, NY, USA) in oxygen and room air under controlled respiration using a ventilator. All imaging examinations were performed in a supine position. The protocol consisted of a sagittal and transverse T1-weighted sequence (repetition time [TR]/echo time [TE] 570/13.8 msec; slice thickness 1.5 mm), T2-weighted sequence (TR/TE 3113/90 msec; slice thickness 1.5 mm), and contrast enhanced T1-weighted sequence after the intravenous injection of 0.1 mmol/kg of gadodiamide hydrate (OMNISCAN, Daiichi-Sankyo, Tokyo, Japan). Diffusion tensor imaging sequences were acquired from the T8-T9 intervertebral disk level to the L2–L3 intervertebral disk level. The protocol consisted of transverse DTI sequences (TR/TE 5571/70 msec; slice thickness 2 mm; diffusion gradient encoding in 32 directions; b value 1,000 sec/mm^2; field of view 250 × 250 mm; scan time 5 min).

Image analysis

Conventional MRI sequences were used to identify relevant anatomy. All measurements were performed by E.N. who was a practicing veterinarian who received training in veterinary radiology and neurology for seven years. FA and ADC maps were created using the package built into the MRI system, and the post-processed DTI images were overlaid with the T2-weighted images to correctly detect the spinal cord. In order to measure the FA and ADC values, eight regions of interest (ROIs) were placed manually at transverse images on each intervertebral disk level slice from T8-T9 through L2–L3 (Fig. 1). All ROIs were set to include more than 18 voxels with special care not to include cerebrospinal fluid as much as possible. All DTI data were analyzed using the workstation built into the MRI system to obtain FA and ADC values. DTI data of the TLSC region (T8–L3 region) were acquired from the nerve fiber bundles of the spinal cord passing through T8–L3, and the ADC and FA values of the entire visualized fibers were averaged and calculated.

Statistical analysis

Statistical analyses were performed using Easy R software [17]. The ADC and FA values were compared among DM, IVDH, and healthy control groups using the Kruskal-Wallis test. Post hoc comparisons employed the Mann-Whitney *U* test with Bonferroni correction. The correlation coefficient (r_s) was calculated by evaluating the correlation between the DTI parameters and clinical duration of DM by Spearman's rank correlation coefficient. We defined clinical duration as the time from the date of clinical onset first noted by the owner to the date of MRI. In all analyses, a *P* value of <0.05 was considered significant. The correlation coefficients of >0.8 were defined as a strong correlation and 0.6–0.8 were defined as a moderate correlation [7].

RESULTS

Sample characteristics

The characteristics of all dogs are shown in Table 1. We included eight DM-affected PWCs in the DM group. Clinical stages of DM were stage 1 in 4 dogs and stage 4 in 4 dogs. Four PWCs, which were categorized as stage 4, underwent MRI within 24 hr of death (Dog #1, 2, 3, 4). These dogs were diagnosed with DM based on histopathological examination of the spinal cord. There was one intact female and three castrated males. The other four PWCs were presumptively diagnosed with DM according to the inclusion criteria. Although four dogs had IVDH lesions, all four dogs had progressive clinical signs at least a year, which were inconsistent with IVDH. Therefore, all four dogs were diagnosed as DM. There were two castrated males and two spayed females. The median age and body weight of dogs in the DM group was 14.1 years (range; 11.0–15.9 years) and 12.1 kg (range; 10.1–14.6 kg), respectively. In the DM group, the locations of IVDHs were T13–L1 (n=4), T12–T13 (n=2), L1–L2, and L2–L3 (n=1). In the IVDH group, we included thirteen dogs diagnosed with thoracolumbar intervertebral disk herniation. Neurological grades were as follows: grade 1 (n=2), grade 2 (n=2), grade 3 (n=3), grade 4 (n=4), and grade 5 (n=2). The locations of IVDHs were T13–L1, L2–L3 (n=6), T12–T13 (n=4), T11–T12, L1–L2 (n=2), and L3–L4, L4–L5, L5–L6, L6–L7 (n=1, each). The number of disk herniation sites in the IVDH group was one (n=8), two (n=2), three (n=1), four (n=1), and five (n=1) (Table 1). There were two intact females, two spayed females, five intact males, and four castrated males. The median age and body weight of the dogs in the IVDH group

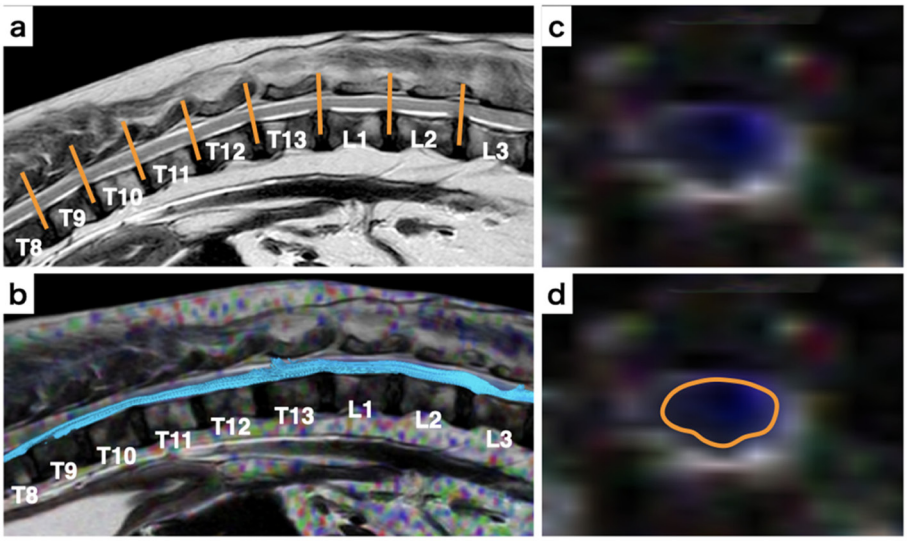

Fig. 1. Fiber tractography and placement of regions of interest (ROI) in the thoracolumbar spinal cord. (Dog #5). (**a**) Eight ROIs were placed manually on transverse T2-weighted images on each intervertebral disk level slice from T8–T9 through L2–L3. (**b**) The axonal bundles of the spinal cord are string-like and appear mainly in blue due to the craniocaudal direction of water diffusion. Axonal bundles were tracked from T8-T9 through to the L2–L3 intervertebral level. (**c**) The T12–T13 intervertebral level slice is shown on a transverse FA map. (**d**) The region of interest is placed on T12–T13 spinal cord area.

Table 1. Characteristics of dogs

Dog number	Diagnosis	Breed	Age (year)	Body weight (kg)	Gender	Location(s) of IVDH	Clinical duration
1	DM-confirmed	PWC	13.0	10.2	CM	T12–T13, T13–L1, L2–L3	3.0 years
2	DM-confirmed	PWC	15.2	10.1	CM	L1–L2	3.5 years
3	DM-confirmed	PWC	14.1	14.6	IF	None	3.3 years
4	DM-confirmed	PWC	15.9	14.6	CM	T13–L1	3.0 years
5	DM-suspected	PWC	14.1	11.8	CM	T13–L1	2 months
6	DM-suspected	PWC	12.7	13.6	CM	T12–T13, T13–L1	3 months
7	DM-suspected	PWC	11.0	12.3	SF	None	7 months
8	DM-suspected	PWC	10.8	16.8	SF	None	2 months
9	IVDH	CB	11.7	3.4	CM	T12–T13	5 days
10	IVDH	Pug	11.6	6.3	SF	T11–T12, T12–T13, L5–L6, L6–L7	3 months
11	IVDH	MD	3.5	5.2	IF	T13–L1	3 days
12	IVDH	MD	5.0	4.2	CM	T13–L1	7 days
13	IVDH	MD	10.8	4.5	SF	T13–L1, L2–L3	4 days
14	IVDH	MD	10.9	5.8	IF	L2–L3, L3–L4	1 month
15	IVDH	TP	5.1	6.7	IM	L1–L2	11 days
16	IVDH	MD	10.7	8.0	IM	L2–L3	10 days
17	IVDH	CB	11.9	5.6	IM	T13–L1	1 month
18	IVDH	MD	9.8	10.7	IM	T13–L1	5 days
19	IVDH	MP	4.7	4.8	CM	T11–T12, T12–T13, L2–L3	5 days
20	IVDH	MD	8.9	5.3	IM	L2–L3	3 days
21	IVDH	MD	10.1	7.1	CM	T12–T13, T13–L1, L1–L2, L2–L3, L4–L5	1 month
22	Hearthy	Beagle	4.0	13.5	SF	None	
23	Hearthy	Beagle	4.0	18.4	CM	None	
24	Hearthy	Beagle	5.0	14.0	CM	None	
25	Hearthy	Beagle	5.5	12.3	SF	None	
26	Hearthy	PWC	10.7	10.7	SF	None	
27	Hearthy	Crossbreed	2.8	10.6	CM	None	

DM, degenerative myelopathy; IVDH, intervertebral disc herniation; PWC, pembroke Welsh Corgi; CB, cross–bred; MD, Miniature Dachshund; TP, Toy Poodle; MP, Miniature Pinscher; CM, castrated male; IM, intact male; SF, spayed female; IF, intact female.

was 10.1 years (range; 3.5–11.9 years) and 5.6 kg (range; 3.4–10.7 kg), respectively. Included breeds were Miniature Dachshund (n=8), crossbreeds (n=2), Toy Poodle (n=1), Pug (n=1), and Miniature Pinscher (n=1). Surgical treatment was performed in 10 dogs, and non-surgical treatment was selected in 3 dogs. All dogs improved their clinical signs. In the healthy control group, we included six dogs. Four dogs were laboratory animals at Gifu University, and two dogs were client-owned dogs. Three laboratory dogs in control group had no structural lesions in the central nervous system. One laboratory dog was diagnosed with idiopathic epilepsy. Two client-owned dogs in the control group had transient limb ataxia but no structural lesions in the central nervous system. There were three spayed female and three castrated males. The median age and body weight of the dogs in the healthy control group was 4 years (range; 4–10 years) and 12.9 kg (range; 10.6–16.8 kg), respectively. Included breeds were Beagle (n=4), PWC (n=1), and crossbred (n=1).

ADC values in DM, IVDH, and control groups

The absolute measurement of DTI parameters and results of statistical analyses were presented in Table 2, Figs. 2 and 3. Among the three groups, there were significant differences in ADC values at T10–T11, T12–T13, L1–L2, and L2–L3 intervertebral disk levels (P=0.024, 0.007, 0.027, 0.017, and 0.007, respectively). The ADC values at T10–T11, T12–T13, T13–L1, L1–L2, and L2–L3 intervertebral disk levels were significantly lower in the DM group than in the control group (P=0.038, 0.004, 0.041, 0.038, and 0.024, respectively). The ADC values at L1–L2 intervertebral disk level was significantly lower in the DM group than in the IVDH group (P=0.038). The ADC values for the T8–L3 region were also significantly different among the three groups (P<0.001). The ADC values for the T8–L3 region were significantly lower in the DM group than in the control and IVDH groups (P=0.002 and P<0.001).

FA values in DM, IVDH, and control groups

Among the three groups, there were no significant differences in the FA values among the three groups in each slice between T8–T9 and L2–L3 intervertebral disk levels. The FA value of the T8–L3 region was significantly different among the three groups (P=0.004). The FA value of the T8–L3 region were significantly lower in the DM group than in the control and IVDH group (P=0.024 and 0.006).

Table 2. Comparison of apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values among degenerative myelopathy (DM), intervertebral disc herniation (IVDH), and healthy control groups

	Intervertebral level	Control		DM		IVDH		*P* value			
		Median	Range	Median	Range	Median	Range	3 groups	Control vs. DM	Control vs. IVDH	DM vs. IVDH
ADC ($\times 10^{-3}$ mm^2/sec)	T8–T9	1.096	0.239–1.567	0.575	0.198–2.287	1.065	0.732–2.457	0.123	1.000	1.000	0.091
	T9–T10	1.338	0.617–1.578	0.536	0.194–1.958	1.162	0.615–1.952	0.134	0.540	1.000	0.230
	T10–T11	1.360	0.913–1.787	0.780	0.107–1.348	1.324	0.605–2.023	0.024*	0.038*	1.000	0.060
	T11–T12	1.339	0.982–2.059	0.836	0.579–1.422	1.263	0.542–1.902	0.062	0.060	1.000	0.360
	T12–T13	1.445	1.243–1.763	0.857	0.519–1.280	1.183	0.746–1.946	0.007*	0.004*	0.537	0.091
	T13–L1	1.285	0.984–1.428	0.748	0.393–1.437	1.305	0.682–1.733	0.027*	0.041*	1.000	0.060
	L1–L2	1.325	0.896–1.473	0.915	0.261–1.171	1.317	0.408–1.603	0.017*	0.038*	1.000	0.038*
	L2–L3	1.360	1.252–1.728	0.892	0.246–1.431	1.116	0.544–2.188	0.007*	0.024*	0.109	0.110
	T8–L3	1.408	1.048–1.627	0.713	0.440–0.840	1.279	0.767–1.767	<0.001*	0.002*	0.969	0.001*
FA	T8–T9	0.574	0.443–0.614	0.547	0.323–0.673	0.548	0.419–0.813	0.901	1.000	1.000	1.000
	T9–T10	0.557	0.427–0.646	0.475	0.352–0.726	0.554	0.388–0.827	0.385	0.820	1.000	0.710
	T10–T11	0.520	0.489–0.612	0.446	0.310–0.750	0.489	0.394–0.843	0.139	0.088	1.000	0.804
	T11–T12	0.520	0.417–0.562	0.460	0.332–0.860	0.552	0.366–0.700	0.145	0.730	1.000	0.230
	T12–T13	0.522	0.460–0.602	0.452	0.341–0.686	0.525	0.361–0.677	0.236	0.430	1.000	0.480
	T13–L1	0.587	0.481–0.626	0.457	0.364–0.669	0.471	0.360–0.633	0.109	1.000	0.053	1.000
	L1–L2	0.603	0.476–0.666	0.506	0.340–0.751	0.468	0.421–0.810	0.195	1.000	0.086	1.000
	L2–L3	0.486	0.275–0.593	0.459	0.356–0.783	0.525	0.359–0.769	0.350	1.000	0.550	1.000
	T8–L3	0.544	0.489–0.576	0.449	0.417–0.550	0.575	0.418–0.701	0.004*	0.024*	1.000	0.006*

*$P<0.05$.

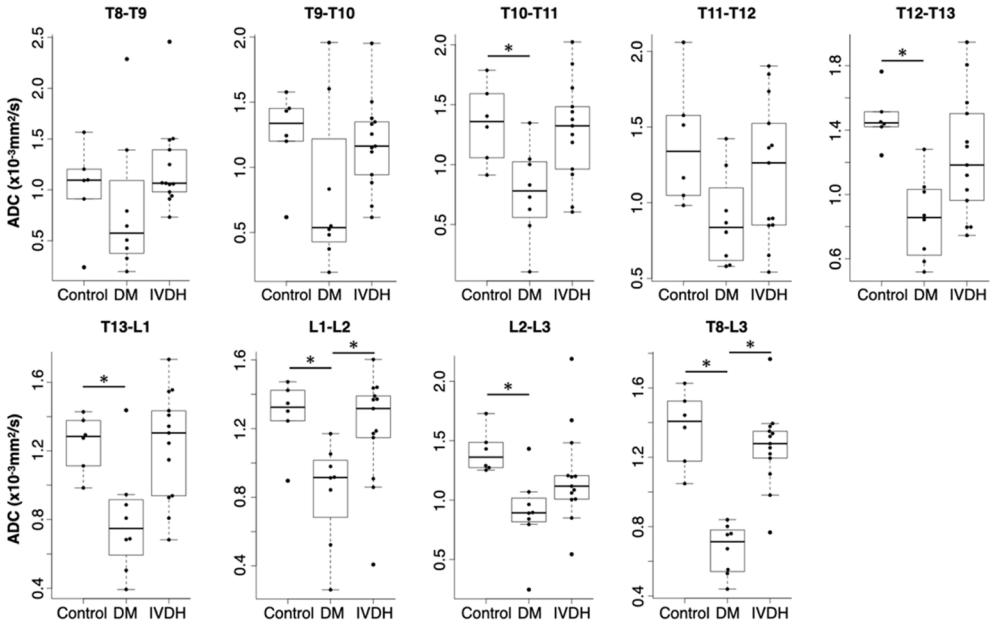

Fig. 2. Comparison of apparent diffusion coefficient (ADC) values among degenerative myelopathy (DM), intervertebral disk herniation (IVDH), and healthy control groups. The ADC values at T10–T11, T12–T13, T13, L1, L1–L2, L2–L3 intervertebral disk levels, and T8–L3 region were significantly lower in the DM group than in the control group (P=0.038, 0.004, 0.041, 0.038, 0.024, and 0.002, respectively). The ADC values at L1–L2 intervertebral disk level and T8–L3 region were significantly lower in the DM group than in the IVDH group (P=0.038 and $P<0.001$). Horizontal bars indicate medians within groups. Statistical analyses were performed using the Kruskal-Wallis test. *Post hoc* comparisons used Mann-Whitney *U* test with Bonferroni correction. *$P<0.05$.

DTI parameters in DM dogs with different clinical duration

The ADC and FA values of DM stage 1 and stage 4 are shown in Table 3. The ADC values at T8–T9, T9–T10, T11–T12, T13–L1, L1–L2, and L2–L3 intervertebral disk levels were found to have strong or moderate negative correlations with clinical

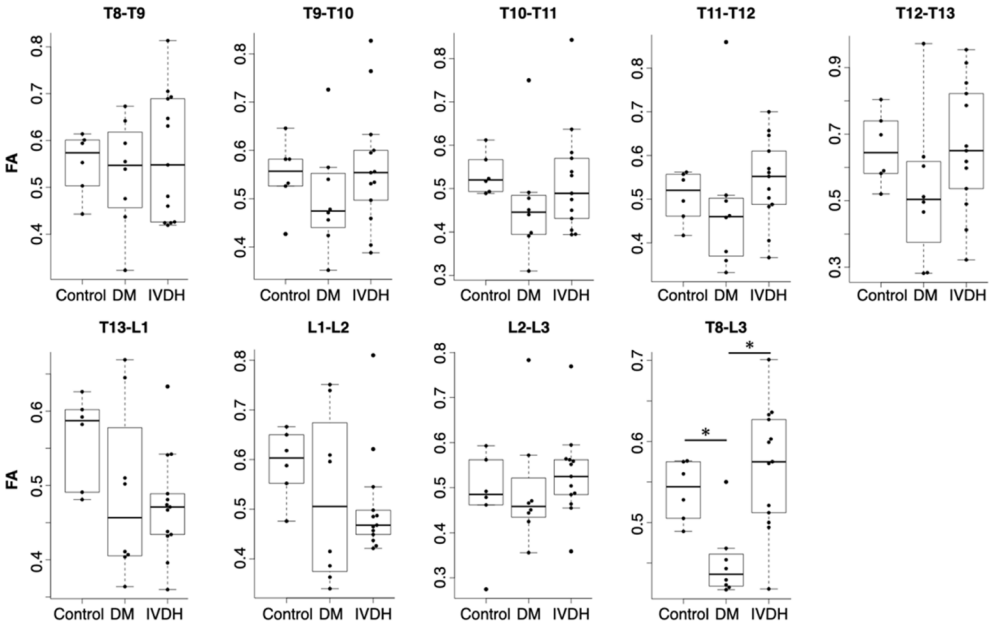

Fig. 3. Comparison of fractional anisotropy (FA) values among degenerative myelopathy (DM), intervertebral disk herniation (IVDH), and healthy control groups. The FA value of the T8–L3 region were significantly lower in the DM group than in the control and IVDH group (P=0.024 and 0.006). Horizontal bars indicate medians within groups. Statistical analyses were performed using the Kruskal-Wallis test. *Post hoc* comparisons used Mann-Whitney *U* test with Bonferroni correction. *P<0.05.

Table 3. Diffusion tensor imaging (DTI) parameters in degenerative myelopathy (DM) stage 1 and DM stage 4

	Intervertebral level	DM stage 1		DM stage 4		Correlation between the clinical duration of DM and DTI parameters	
		Median	Range	Median	Range	r_s	P value
ADC	T8–T9	1.018	0.426–2.287	0.417	0.198–0.792	–0.711	0.048*
	T9–T10	1.076	0.482–1.958	0.448	0.194–0.832	–0.711	0.048*
	T10–T11	0.916	0.627–1.348	0.611	0.107–1.047	–0.470	0.240
	T11–T12	1.097	0.649–1.422	0.696	0.579–0.867	–0.711	0.048*
	T12–T13	0.930	0.662–1.280	0.727	0.519–1.046	–0.313	0.450
	T13–L1	0.916	0.808–1.437	0.594	0.393–0.688	–0.873	0.011*
	L1–L2	1.016	0.917–1.171	0.683	0.261–0.913	–0.783	0.022*
	L2–L3	1.016	0.889–1.431	0.818	0.246–0.894	–0.747	0.033*
	T8–L3	0.781	0.754–0.840	0.541	0.440–0.672	–0.723	0.043*
FA	T8–T9	0.488	0.323–0.555	0.618	0.476–0.673	–0.819	0.013*
	T9–T10	0.522	0.424–0.726	0.464	0.352–0.540	–0.325	0.432
	T10–T11	0.485	0.398–0.750	0.412	0.310–0.451	–0.518	0.188
	T11–T12	0.486	0.380–0.860	0.409	0.332–0.496	–0.446	0.268
	T12–T13	0.452	0.433–0.686	0.422	0.341–0.516	–0.036	0.932
	T13–L1	0.457	0.404–0.669	0.459	0.364–0.645	0.084	0.843
	L1–L2	0.512	0.386–0.751	0.480	0.340–0.739	–0.133	0.754
	L2–L3	0.448	0.356–0.572	0.459	0.444–0.783	0.253	0.545
	T8–L3	0.456	0.420–0.550	0.426	0.417–0.454	–0.446	0.268

*P<0.05; ADC, apparent diffusion coefficient; FA, fractional anisotropy.

durations, especially the ADC value at T13–L1 was found to have the strongest negative correlation (r_s= −0.873, P=0.011). The ADC values for the T8–L3 region had a moderate negative correlation with clinical duration (r_s= −0.723, P=0.043). The FA value at T8–T9 intervertebral disk level had a strong negative correlation (r_s= −0.819, P=0.013), but the FA values of other intervertebral disk levels and T8–L3 region had no correlation with clinical durations.

DTI parameters in IVDH dogs with different clinical grades

In all intervertebral disk levels and T8–L3 region, the ADC values had no significant correlation with clinical grades of IVDH. Similarly, The FA values of all intervertebral disk levels and T8–L3 segment had no significant correlation with clinical grades of IVDH.

DISCUSSION

The present study revealed that ADC and FA values of the T8–L3 region were significantly decreased in DM group compared with those in control and IVDH groups. Especially, ADC value of T8–L3 region may be a potential biomarker for distinguishing DM from IVDH. Moreover, ADC values were negatively correlated with clinical grades in DM. As neurodegeneration of the spinal cord in dogs with DM is not visualized by conventional MRI sequences, DTI parameter has a potential to be used as a non-invasive diagnostic test for DM.

In this study, the ADC value of the T8–L3 region in the DM group was significantly lower than that in the IVDH group. The ADC value is a parameter obtained based on restriction of the movement of extracellular water molecules. In humans, it is used for diagnosis, treatment monitoring, and assessing the prognosis of spinal cord diseases such as ALS [14, 30, 32], cervical spondylotic myelopathy [5, 11], compressive myelopathy [21, 38], multiple sclerosis [4, 19], and spinal cord injury [34]. In spinal cord diseases in dogs, ADC values in the lesions of the thoracolumbar disk herniation and meningioma are lower than those in the healthy areas [20]. Thus, it is generally considered that the decrease in ADC value is due to the influence of the change in water content caused by cytotoxic edema and the change in lipid content due to demyelination, thereby reducing the degree of diffusion of water molecules [12]. Furthermore, histiocytic sarcoma, which is clinically more malignant than brain meningiomas in dogs, was reported to have a lower ADC level than meningioma [37]. As a tumor with high malignancy has a high cell density, a higher cell density results in a lower ADC value. In DM-affected PWC, demyelination in the thoracolumbar spinal cord, proliferation of fibrous tissue, microgliosis, and diffuse proliferation of astrocytes are observed [3, 9, 23, 25]. Also, the space caused by axonal loss and demyelination were replaced by homogeneous eosinophilic matrix associated with astrocytosis [23]. These pathological features suggest that the demyelination and increased cell density in the thoracolumbar spinal cord of DM-affected dogs restrict the movement of water molecules and reduce ADC levels. It is necessary to evaluate the relationship between ADC values and histopathological findings in future studies.

ADC values of L1–L2 intervertebral disk levels in the DM group were significantly lower than those in the IVDH group, while there were no significant differences between the two groups in ADC values at other intervertebral disk levels. In contrast, a previous report demonstrated that IVDH lesions had lower ADC values than normal regions of the spinal cord [20]. In general, ADC values increase in the spinal cord with edema and/or demyelination secondary to IVDH whereas significant increase of ADC values is not seen in the spinal cord without edema/demyelination [12]. Therefore, it is difficult to quantify the degree of spinal cord degeneration under the influence of compressive lesions, and it is necessary to evaluate multiple spinal cord segments including a spinal cord region not unaffected by IVDH.

In this study, the FA value of the T8–L3 region in DM group was significantly lower than that in the IVDH group. The FA value is a quantification of the degree of anisotropy and the decrease in diffusion anisotropy reflects the incompleteness of nerve fiber bundles in the spinal cord [31]. In spinal cord diseases in dogs, the FA value is lower in chronic spinal cord injury lesions than in the healthy area [37]. In chronic spinal cord injury, axonal degeneration and demyelination are considered to impair the integrity of nerve fiber bundles and reduce FA levels. In PWC with DM, marked axonal degeneration and demyelination are found in the white matter of the thoracolumbar spinal cord [3, 9, 23, 25]. This suggested that degenerative changes in the spinal cord white matter in DM-affected dogs caused the impaired nerve fiber bundles and reduced FA levels. Currently, the association between FA levels and histological changes in the spinal cord of dogs with DM is unknown. Therefore, it is necessary to evaluate the association between FA values and histopathological findings in future studies.

In chronic spinal cord injury in dogs, the FA value in the lesioned area decreases and is further correlated with the clinical score [22]. As the IVDH group in this study contained dogs with varying grades and herniated sites, there was no significant difference among the three groups in FA values at each intervertebral level. A previous report revealed that the DM group showed significantly lower FA values than the healthy group when assessing FA values using the cranial lumbar region of the spinal cord [15]. Although, there was no significant difference when assessing FA levels at the disk level in the present study, there was a significant difference in the broader spinal cord assessment. Since widespread white matter degeneration is seen in the spinal cord of DM, a wide measurement range may be required to detect changes of the degenerative lesions.

When comparing the ADC and FA values at T8–L3 region in the DM-suspected group and DM-confirmed group, ADC value was negatively correlated with clinical duration; however, there was no significant difference in FA value. In general, the ADC value decreases due to restriction of the movement of water molecules in proportion to the degree of cytotoxic edema and increased cell density [12]. In the thoracolumbar spinal cord of dogs with DM, the degree of extracellular matrix and cell density is considered to increase due to glial cells depending on the stage of disease [3, 9, 23, 25]. Therefore, water molecule movement may be gradually restricted. On the other hand, axonal degeneration and demyelination of the white matter in the thoracolumbar spinal cord have been observed since early stage of DM [10, 23]. This histopathological finding supports the isotropic diffusion of water molecules in the thoracolumbar spinal cord from the early stages of DM. However, the recent DTI study reported that FA values were significantly correlated with histopathological grade of the DM spinal cord [15]. This different result is considered to be caused by the difference in the measurement site. Further studies are needed on the relationship among the clinical stage,

histopathological findings, measurement site, and MRI parameters.

The results of the present study showed that the ADC and FA values of the T8–L3 region were superior to those of cross-sectional spinal cord to assess spinal cord degeneration in DM. In ALS studies, using a 9.4-Tesla MRI system, the axial diffusion and radial diffusion values of the gray matter of the spinal cord were reduced in ALS model mouse group compared to healthy control group [13]. In another study, FA values obtained from the corticospinal tract were reduced in ALS patient group compared to healthy control group [6]. In dogs of which the size of the spinal cord is much smaller than humans, it is difficult to set an ROI to target only a specific region of the spinal cord, using a clinical MRI system. On the other hand, radial diffusion and FA values obtained not only from a single spinal cord segment but multiple spinal cord segments were reduced in ALS group compared to healthy controls [26, 30]. Therefore, evaluation of DTI parameters using multiple spinal cord segments (T8–L3 region) was considered to be suitable for capturing spinal cord degeneration in dogs. In the future, it is necessary to investigate the region of spinal cord segments with the highest diagnostic ability for detecting DM lesions.

There are several limitations in this study. Measurements of ADC and FA values were performed and analyzed by a single observer. Further study is needed to evaluate intra- and inter-observer errors. In the DM group, MRI data for four dogs were obtained postmortem in order to compare the difference of DTI parameters between the early stage and late stage. This comparison was only possible by using postmortem MRI as dogs with DM in the late stage suffer from respiratory disfunction that hinders diagnostics requiring general anesthesia. DTI data for 4 cases in the DM group were obtained postmortem. A previous study using mice reported that DTI data obtained 10 hr after death were comparable with those obtained premortem [18]. Postmortem MRI was performed within 24 hr of death in an attempt to minimize any postmortem changes. Postmortem changes need to be investigated in terms of their effects on histopathological changes and DTI data. Further, the degree of spinal cord degeneration was not quantified by histopathological examination and the correlation between histopathological changes and DTI parameters were not investigated. Four dogs were tentatively diagnosed with DM based on our diagnostic criteria without histopathological confirmation. These four dogs were still alive at the time of manuscript preparation; therefore, this study could not compare the histopathological findings of DM with MRI. The sample size was small, and the age, weight, and breed in each group were not consistent. Specific diagnostic parameters such as the cut-off value, sensitivity, and specificity need to be determined upon increasing the numbers of dogs in each group. The movement of water molecules may be affected by breathing, heartbeat, cerebrospinal fluid pulsation, and anesthesia [36]. CSF and surrounding tissue may also be included due to the limited ROI resolution. Using the average ADC value and FA value of the target areas visualized by fiber tractography may reduce these artifacts.

In conclusion, quantitative data using DTI may be the potential biomarker for distinguishing DM from IVDH. In particular, the ADC value of the T8–L3 region may aid in the premortem diagnosis of DM.

CONFLICT OF INTEREST. None of the authors have a financial or personal relationship with other persons of organizations who can inappropriately influence or bias the content of the paper.

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