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ULTRASOUND ASSESSMENT OF THE DIAPHRAGM: PRELIMINARY STUDY OF A CANINE MODEL OF X-LINKED MYOTUBULAR MYOPATHY

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Abstract

Introduction—We tested the feasibility of using neuromuscular ultrasound for non-invasive real time assessment of diaphragmatic structure and function in a canine model of X-Linked Myotubular Myopathy (XLMTM).

Methods—Ultrasound images in 3 dogs (Wild Type WT, n=1; XLMTM untreated, n=1; XLMTM post AAV8-mediated MTM1 gene replacement, n=1) were analyzed for diaphragm thickness, change in thickness with respiration, muscle echogenicity, and diaphragm excursion amplitude during spontaneous breathing.

Results—Quantitative parameters of diaphragm structure were different among the animals. WT diaphragm was thicker and less echogenic than the XLMTM control, whereas the diaphragm measurements of the MTM1-treated XLMTM dog were comparable to the WT dog.

Discussion—This pilot demonstrates the feasibility of using ultrasound for quantitative assessment of the diaphragm in a canine model. Ultrasonography may potentially replace invasive measures of diaphragm function in canine models and in humans in the future, for non-invasive respiratory monitoring and evaluation of neuromuscular disease.

Keywords

canine; diaphragm; respiratory failure; ultrasonography; ultrasound

INTRODUCTION

Electromyographic needle examination is the prevailing method for diaphragm muscle assessment. In animals, it can be challenging due to small, thin diaphragms and the risk of injury due to inaccurate needle placement.¹ Other methods for assessing diaphragms include respiratory inductive plethysmography,² piezoelectric contact sensors placed on the costal wall,¹ sonomicrometry,^{3,4} or transdiaphragmatic pressure measurements.⁵ Limitations of these methods include the need for training and specialized equipment, and most are invasive and cumbersome.

Ultrasonography can provide non-invasive, radiation-free, direct, and real-time visualization of anatomic structures, and it is now being used to assess muscle pathology. Advances in ultrasound technology have enabled acquisition of high resolution images.^{6,7} Quantitative parameters of muscle ultrasound have proven use in evaluation of muscle pathology.⁸ Here we report the use of neuromuscular ultrasound for assessment of diaphragm structure and function in a canine model of myotubular myopathy.

MATERIAL AND METHODS

The Animal Care and Use Committee of Wake Forest School of Medicine approved all experiments in live animals. Ultrasound (ACUSON Sequoia 512 Ultrasound System, Siemens, US™) was used to evaluate the diaphragm in spontaneously breathing, age-matched anesthetized dogs (Wild type-WT: n=1; X Linked Myotubular Myopathy-XLMTM: n=2). One XLMTM dog received a single intravascular injection of an AAV8 vector carrying a canine cDNA for MTM1 (2.5×10^{12} vector genomes per kg body weight) 4 weeks prior to assessments.⁹

A 15 MHz linear array transducer was placed in the lowest intercostal space to visualize the diaphragm in the zone of apposition through the liver window using the lateral intercostal approach in the mid-axillary line (Supplemental Figure 1A, available online).⁷ All lower intercostal spaces were evaluated to identify the space with the best visualization of the diaphragm, where the muscle is thickest and with minimal encroachment of the lung during inspiration.⁷ Using a standard intercostal space did not allow end-inspiration muscle to be captured in all beagles due to different body sizes and different tidal volumes during spontaneous ventilation. Images were acquired using B-mode ultrasound to measure diaphragm thickness at end-expiration and end-inspiration. In addition, to allow visualization of the dome of the diaphragm for excursion amplitude, a lower frequency 4 MHz probe was placed in the right subcostal space in the mid-clavicular line (Supplemental Figure 1B). Images were acquired using the M-mode of ultrasound to assess the amplitude of diaphragmatic excursion during spontaneous breathing.⁷ Doxapram, an intravenous respiratory stimulant, was administered and measurements were repeated to assess feasibility of quantitative measurements at higher respiratory rates. Echodensity of the muscle was measured using ImageJ® software by creating a gray scale analysis histogram on outlined muscle cross-section (Supplemental Figure 1C and 1D).¹⁰ Muscle echodensity on gray scale analysis have been reported to correlate with muscle pathology.¹¹ Velocity of diaphragm contraction was derived by dividing the excursion amplitude by end-expiration to

peak inspiration time. Diaphragm excursion and velocity are being explored as surrogates of diaphragm function. While excursion of the diaphragm may occur even in a paralyzed diaphragm, velocity of the diaphragm contraction may reflect diaphragm strength during spontaneous breathing hence was separately measured.

RESULTS

On B-mode images, each rib was identified by the bright signal generated at its bony cortex and the acoustic shadowing deep to it. Two layers of intercostal muscle were seen to bridge the space between adjacent ribs. Diaphragm was identified by its location, curved geometry, and muscle echo texture (Figure 1A). The diaphragm muscle layers appeared as 2 echogenic layers of peritoneum and pleura sandwiching a more hypoechoic line of the muscle itself that moved and thickened during inspiration due to muscle contraction.^{12, 13} On M-mode imaging, the diaphragm tendon was isolated as a curvilinear single-layer echogenic structure seen through the liver window (Figure 1B).

Quantitative data are listed in the table. The WT diaphragm was thicker and less echogenic than the untreated XLMTM diaphragm ($3.69\text{--}2.73$ mm and 56.839 ± 10.71 vs. $1.2\text{--}1.01$ mm, and 86.471 ± 13.34 , respectively), whereas the treated XLMTM diaphragm ($3.5\text{--}1.93$ mm and 45.254 ± 10.37) was comparable to the WT diaphragm (Supplemental Figure 2). WT diaphragm showed a trend toward an increase in diaphragm excursion amplitude and velocity after respiratory stimulant administration. XLMTM diaphragm showed a trend toward increase in velocity, while treated XLMTM showed a trend toward decrease in excursion.

DISCUSSION

Muscle ultrasound changes have been shown to correlate with pathological changes seen on muscle biopsy.^{14,15} Dystrophic muscle becomes thinner and more echogenic as it is replaced by fat and fibrous tissue. Determination of muscle echodensity on gray scale analysis can discriminate between neuromuscular and non-neuromuscular diseases.¹⁶ Studies on diaphragm muscle thickness, excursion, and velocity are being done to explore ultrasound as a non-invasive clinical tool to assess diaphragm structure and function in clinical studies in human subjects.^{7, 17–19} Diaphragm evaluation using ultrasonography has been described in veterinary medicine,^{6,20} but we are unaware of any report using this modality to distinguish diaphragmatic pathology in a canine neuromuscular disease model. Hence, we used quantitative ultrasound to distinguish diaphragm muscle involvement in a myotubular myopathy model.

The canine model of XLMTM provides an opportunity to study large animals with rapidly progressive and fatal muscular phenotypes.^{21–24} XLMTM dogs develop progressively fatal skeletal and respiratory muscle weakness with diaphragm atrophy. We compared ultrasound images of diaphragm acquired from WT beagle with images from XLMTM control and a beagle that received Myotubular Myopathy1 (*MTM1*) gene replacement therapy.⁹ Our findings suggest that thickness and echodensity reflect a functional response to *MTM1* gene replacement in *MTM1*-mutant diaphragm muscle. This observation aligns with recent

findings of a marked increase in peak inspiratory flow following gene replacement in this model.⁹ Higher baseline excursion in XLMTM animal could have been a compensatory adaptation to global respiratory muscle weakness. The trend of changes seen in diaphragm excursion and velocity are of unclear significance and suggest that these 2 parameters may not correlate with diaphragm function. Definitive interpretation was limited by sample size and lack of clinical markers of lung function, but quantitative markers obtained via muscle ultrasound may be useful for evaluation of diaphragm muscle pathology in a canine model.

Conclusion

Ultrasonography as a non-invasive technique has the potential to replace or supplement invasive measures of diaphragm muscle function in canine models and in the future could provide a clinically useful method to assess diaphragm function in patients with neuromuscular disorders.

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ABBREVIATIONS

WT	wild type
XLMTM	X linked Myotubular Myopathy
MTM	Myotubular Myopathy

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Figure 1.

A) Diaphragm visualized in the zone of apposition through the liver window with the lateral intercostal approach in mid-axillary line using a 15 MHz probe. B) Dome of the diaphragm visualized in the right subcostal space in the mid-clavicular line using a 4 MHz probe. C and D) Echodensity of the muscle was measured using ImageJ® software by creating a gray scale analysis histogram on outlined muscle cross-section area.

Figure 2.

A) Healthy Beagle Wild Type (WT). B) Beagle affected by XLMTM after experimental gene therapy. C) Beagle affected by X-linked Myotubular Myopathy

Table 1

Quantitative parameters of the diaphragm structure assessed using ultrasound

	Thickness (mm)			Echodensity [†]		Excursion [‡] (mm)		Velocity [§] (mm/s)	
	Max	Min	Change (%) [*]	Mean	SD	Before	After 10 min	Before	After 10 min
WT	3.69	2.73	26.01	56.839	10.71	9.94	13.00	41.76	53.85
XLMTM	1.2	1.01	15.83	86.471	13.34	19.18	20.80	40.99	67.03
XLMTM-Tx	3.5	1.93	44.85	45.254	10.37	8.8	7.22	40.1	39.24
									36.3

* Change % = (max thickness – min thickness)/max thickness × 100

[†] On Gray Scale, Analysis done in ImageJ®[‡] Before, immediately after, and 10 minutes after doxapam[§] ND – Not determined due to early awakening and interruption of mechanical ventilation in the beagle

WT – Wild type; XLMTM – X-linked Myotubular Myopathy; XLMTM-Tx – XLMTM after gene therapy