MAGNETIC RESONANCE IMAGING, ULTRASONOGRAPHIC AND BIOMECHANICAL EVALUATION OF DIGITAL FLEXOR TENDONS IN DONKEYS (EQUUS ASINUS)

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ABSTRACT

The present study was designed to carry out comparative evaluation of normal digital flexor tendons in donkeys based on Magnetic resonance imaging (MRI), ultrasonography and biomechanical properties. Five clinically healthy donkeys were assigned for ultrasonographic examinations of the metacarpus/metatarsus in each limb. These donkeys were euthanized later for MRI and biomechanical examinations. The biomechanical data was compared and statistically analyzed. Ultrasonographically, the SDFT was slightly less echogenic than DDFT especially at the proximal metacarpal and metatarsal regions; SDFT usually locate above DDFT in the forelimb; however, in the hind limb the two tendons may be nearly parallel to each others. MRI revealed very low signal intensity tendons which appeared black on the image with the cross sectional shape of DDFT is more rounded than SDFT. Biomechanical testing (load failure, strain and stress) showed significant difference (p< 0.05) between both tendons and in both fore and hindlimbs. MRI, ultrasonography and biomechanical evaluation of digital flexor tendons in donkeys provide a useful information about the normal anatomical features of these structures and help in understanding tendon injuries or repair.

Keywords: *Donkey; Digital flexor tendons; MRI; Ultrasonography Biomechanics.*

INTRODUCTION

Knowledge of normal anatomy of equine limbs is essential for understanding digit deformities or injuries and is crucial for accurate interpretation of diagnostic images as seen with ultrasonographic examination and magnetic resonance imaging (MRI) (**Denoix, 1996**).

Ultrasonographic imaging of equine flexor tendons provides safe noninvasive means of anatomical and lesions diagnosis (**Nyland and Matton, 1995**). For accurate diagnosis of equine tendons, two sonographic views are performed especially under weight bearing situation : one sagittal, to observe the normal alignment of the tendon fibers, and the other one is transverse, to obtain structural dimensions (**Howell, 2005**).

MRI is a gold standard imaging modalities accurately diagnose tendons architectures and lesions which were previously unable to be visualized with radiography and ultrasonography **(Kletter et al., 1999; and Kraft & Gavin, 2001)**. MRI can be acquired in any desired orientation but commonly acquired in transverse, sagittal, and dorsal planes. The number and thickness of the slices can be varied to cover a region of interest or focus on a specific structure **(Tucker and Sande, 2001; and Werpy, 2007)**.

Biomechanical parameters of the equine SDFT, DDFT and SL include the ultimate tensile stress, ultimate tensile strain, and the modulus of elasticity **(Smith and Webbon, 1996)**. The tensile stress of tendons is related to thickness and collagen content. A tendon with an area of 1 cm^2 is capable of bearing 500 to 1000 kg of load **(Riemersma et al., 1996)**.

Strain was defined as the change in length of a substance normalized by the original length **(Becker et al., 1994)**. While, stress is fundamentally simple to measure, where a constant load is applied to a tissue and the progressive time dependant elongation is measured **(Holmstrom et al., 1994)**. However, load to failure represent the continuous loading of a tendon sample till complete rupture **(Woo et al., 1997)**.

Use of biomechanical properties and MRI to evaluate normal morphological features of digital flexors in donkeys has not been previously described. Therefore, the present study was designed to evaluate the role of MRI, ultrasonographic and biomechanical properties of normal digital flexors tendons in donkeys as well as their identification and correlation at the different regions of the limb.

MATERIALS AND METHODS

A total of 5 clinically healthy donkeys at the same ages (5 years) weight about 140-180 kg and free from locomotor disorders; were used for this study. These animals were firstly subjected to ultrasonographic examinations at different levels of the fore- and hindlimbs. The second division of this study was applied on the limbs of donkeys after euthanasia to serve as cadavers for MRI (5 forelimb and 5 hindlimb) and biomechanical evaluation (5 forelimb and 5 hindlimb) of digital flexors in both metacarpal and metatarsal regions.

Ultrasonographic evaluation :

Ultrasonographic examination of donkeys limb was carried out using 7.5 MHz mechanical linear scanner (MINDRAY DP-2200Vet., China). For this purpose, the limbs were prepared by clipping, shaving of the hair and application of ultrasound transmission gel at the examined area. Scanning of the limb was done at weight bearing position from just distal to the carpal and/or tarsal joint to the level of the proximal sesamoid bones. Longitudinal and transverse scans were obtained for the examined tendon at three levels equally divided (upper, middle and lower region) of the metacarpus / metatarsus **(Genovese et al., 1997)**. The cross sectional area (CSA) of the digital flexors and their correlation in both fore-and hindlimbs were measured.

Magnetic resonance imaging :

MRI of digital flexors was performed on 10 digit cadavers of the euthanized donkeys at the radiology department at Mansoura

University Teaching Hospital (MUTH). A human extremity radiofrequency receiving coil was placed on the cadaver digit to be imaged, and the magnet was positioned in a manner that placed the digit centrally within the magnetic field. Pilot scans of short duration were performed to determine proper positioning and for purposes of establishing proper angle for the scanning sequences. A standard protocol for foot imaging using 0.5 Tesla FLEXART TOSHIBA magnet that include the following sequences: gradient echo T1-weighted, fast spin echo T2-weighted, and short time inversion recovery (STIR) sequences in sagittal, transverse, and frontal planes with a slice thickness 4mm.

Biomechanical Evaluation :

Intact digital flexor tendons specimens in full thickness of 15 cm in length were taken from the limbs of euthanized donkeys after anatomical dissection. Biomechanical properties were carried out at laboratory of Biomechanics, Faculty of engineering, Mansoura University. These specimens were packed in containers of refrigerated normal saline. Tensile testing occurred within three hours of tissue collection. Specimens were loaded in a hydraulic tensile testing device (LLOYD) by securing its proximal and distal portions to two metal clamps of the Tensometer. The clamps were coated from inside by a piece of felt and tightened to avoid slipping of the tendon specimens. All specimens were loaded to failure (complete rupture) with a 1000 kg load cell moving at a crosshead speed of 500 mm/min. Load trials to failure (failure stress) were recorded using a digital monitor connected to the load frame and graphically by a digital camera. Tendon strains were constantly monitored during loading trials and calculated graphically from the digital video recording **(Lochner et al., 1980)**.

Statistical Analysis :

The obtained data were statistically analyzed with statistical software program (Graph pad prism version 5.0, USA). The mean values and standard deviation (SD) were calculated for biomechanical parameters. Differences between means at $P < 0.05$ were considered significant.

RESULTS

Ultrasonography of normal digital flexors:

Ultrasonography of normal flexor tendons at the proximal, middle and distal third of the metacarpus/metatarsus were identified SDFT, DDFT, ICL and SL. It was characterized by the presence of clear linear echoic bands parallel to each others which divided by intermittent lines exhibiting the acoustic impedance difference between tendons (Fig. 1 - 4).

SDFT has a homogenous and echogenic appearance and is slightly less echogenic than DDFT. It composed of long parallel fiber bundles that appear as long white echoes in the sagittal or long axis view and as a uniform distribution of pinpoint white echoes in the transverse or short axis view. The cross sectional area of SDFT in the metacarpal/metatarsal region ranged from 0.8-1.2 cm^2. In the distal third of metacarpal/metatarsal the homogenicity of SDFT has a thin, half moon shape in the transverse plan. There was ultrasonographic relation between SDFT and DDFT especially at the proximal metacarpal and metatarsal regions; SDFT usually locate above DDFT in the forelimb. However, in the hind limb the two tendons may be nearly parallel to each others (Fig. 5).

DDFT has a homogenous and echogenic appearance, usually appears more echogenic than SDFT, but may be isoechoic with SDFT. It appears sonographically as long white echoes in the sagittal or long axis view and a homogenous distribution of pinpoint white echoes in the transverse or short axis view. DDFT has an oval appearance proximal to the fetlock at the distal third of metacarpal/metatarsal. The cross sectional area of DDFT in the metacarpal/metatarsal region ranged from 0.9-1.6 cm^2.

ICL of DDFT has a homogenous and echogenic appearance and may be appear as the most echogenic structure in the proximal metacarpal/metatarsal region. The echo pattern of ICL is unchanged from that of SDFT and DDFT. The SL origin and body appears more heterogeneous and hypoechoic than other tendinous and ligamentous structures.

MRI of normal digital flexors

Normal digital flexor tendons viewed with MRI showed very low signal intensity and appeared black on the image. They forming a lattice appearance on the transverse view. SDFT has a uniformly low signal intensity until close to its insertion where it becomes more heterogeneous. It contains septa of uniform intermediate signal intensity. As it confirms to the DDFT, the SDFT forms a crescent shape when viewed transversally, with a concave dorsal surface and convex palmer/plantar surface. They are encased in tendon sheath at the proximal and distal aspect of the metacarpal/metatarsal. These synovial sheaths produce areas of high signal intensity surrounding the low signal tendons.

The signal intensity of DDFT in the metacarpal/metatarsal region is quite similar to the SDFT, although the cross sectional shape is more rounded. DDFT normally has a uniform, low signal intensity (hypointense) with tendon fascicles separated by lines of higher signal and difficult to be distinguished from the hypointense SDFT especially at the level of fetlock joint. It becomes bi-lobed in the pastern region; the medial and lateral lobes change in shape from proximally to distally, but are symmetrical in size and shape. As it approaches the navicular bone, it loses its bi-lobed appearance and diverges into a thin band to insert on the palmar aspect of the os-pedis.

ICL appears normally as a thin band of moderately low signal intensity (gray-black) throughout most of its length. Normal SL is composed of low signal intensity (black) surrounded by a thin layer of high signal intensity (white) due to fascia, connective tissue, and adipose tissue surrounding the SL. Proximal SL is almost bi-lobed structure that contains a cleft of high signal intensity connective and adipose tissues. As the SL travel distally the cleft recedes (Fig. 6).

Biomechanics of normal digital flexors:

On loading to failure, all tendons segments were ruptured mainly through the proximal attachment of the tendon segment to the Lloyd arm (Fig. 7). The results of the biomechanical testing (load failure, strain and stress) of normal SDFT and DDFT showed significant difference ($p < 0.05$) in parameters of tensile strength between both tendons and in both fore- and hindlimbs. The influence of strain on fore- and hindlimb in the present study, showed a significant increase in the SDFT and DDFT in the hindlimb as shown in table 1.

Table 1. Means ± SD of the biomechanical properties of normal SDFT and DDFT in both forelimb and hindlimb.

Biomechanical properties	Forelimb		Hindlimb	
	SDFT	**DDFT**	**SDFT**	**DDFT**
Load (N)	5200 ± 860	8500 ± 386	6150 ± 713	9100 ± 295
Strain (%)	10 ± 1. 2	15 ± 2.03	11 ± .9	16 ± 2.00
Stress (N/mm^2)	104 ± 4.7	143 ± 8.1	106 ± 3.5	151 ± 7.8

Significant difference ($p < 0.05$)

DISCUSSION

Under clinical condition, knowledge of normal anatomy of the equine limb is critical for accurate diagnosis of tendon and ligaments injuries **(Denoix et al., 1996)**. Tendons or ligaments normally change in size and shape from their origin to insertion. Because of this variation, equine veterinarians appreciate specimens which demonstrate important anatomical structures **(Latorre et al., 2001)**.

A collaboration between MRI and ultrasonography as diagnostic imaging used to improve the tendon and ligament images will provide an excellent tools for the objective assessment of the effectiveness of the digital flexor tenorrhaphy with different tendon grafts in horses, monitoring of the repair trajectory as it provide intra-vital method of investigation, allow more adequate treatment selection and timely adjustment of therapies or rehabilitation procedures and it may even provide a basis for better early prognostication **(Zhang et al., 2001; Werpy, 2006; and Zubrod & Barrett, 2007)**.

Tendon failure may occur as a single strain event or as cumulative fatigue failure as a result of cyclical loading. Measurement of the digital flexor tendons biomechanical properties may be a more important indicator of tendon strength **(Dowling et al., 2002)**.

Ultrasonographic examination was performed in weight bearing donkeys because when they were not weight bearing; the tension decreased in the tendons and ligaments and there were a reduction in the echogenicity, shape and size which produces artifacts mistaken for an injury **(Micklethwaite et al., 2001)**.

With the traditional zone method, the metacarpal and metatarsal regions are divided into 6 and 8 zones, respectively **(Genovese et al.; 1987)**. These regions are not clearly defined, unless the distance to a reference point is measured. We chose to make a transverse scan at three definitive equal regions (i.e. the upper, the middle, and the lower parts) along the length of the palmer metacarpus or planter metatarsus. It makes the scan more accurate and concise and more accordant with the routine work. Actually, creating broader regions is a problem with measuring, because going up and down the limb a centimeter or two results in a change in CSA, even at rest. These results were in accordance with **Ying-Ling and Ming-Lai (2005)**.

Each tendon and ligament in the metacarpal/metatarsal region normally change in size and shape from its origin to its insertion. Therefore, a through knowledge of the anatomy of the structures under investigation and their interrelationships to one another is crucial for the accurate interpretation of the sonogram **(Majid, 2008)**.

The most important criteria for the ultrasonographic diagnosis of tendon and ligament injuries are changes in echogenicity and size. The SL was the most echogenic structure while the SDFT was the least echogenic. This could be attributed to the variation in the histological structures of the tendons and ligaments; as the SL is predominantly a strong ligamentous band containing variable amounts of muscular tissue and fat **(Dyson, 1998; and Birch et al., 1999)**.

The tendon CSA at the identical level in each of the controlateral fore and hindlimbs should be the same. Sonographically, the CSA of the SL was the largest and that of the SDFT was the smallest at all levels. This findings is related to collagen contents, which is proportional to the CSA. The collagen content of the SL is much smaller than that of the SDFT, possibly due to the presence of fat and some muscle fibers in the SL **(Smith et al., 1994; and Majid, 2008)**.

MRI examinations was conducted on limb cadavers. This may have influenced overall image quality in the different systems because of positioning, absence of motion and blood flow; as motion artifacts may compromise sequences with relatively acquisition time **(Dyson et al., 2003; and Mair et al., 2005)**.

The metacarpal and metatarsal regions are typically examined using dorsal, sagittal and transverse slice planes. This provides the most complete evaluation of all structures in these regions. MRI of the metacarpal and metatarsal regions of the horse is important as a diagnostic aid in detailed assessment of pain causing lameness localized to this area **(Sampson and Tucker, 2007)**.

MRI of normal tendons and ligaments in donkeys revealed individual characteristic shapes and are normally low signal intensity structures. Changes in the normal shape or signal intensity are indications of pathology. These results were agreed with **Zubrod and Barrett (2007)** who mentioned that changes in normal shape or signal intensity with MRI are indications of pathology.

At comparable limb loading, the results of biomechanical testing showed significance difference between the SDFT and DDFT in values of absolute load, strain, and stress at tendon failure. The difference between tensile strength of SDFT and DDFT It is suggested that there was a proportional relationship between the load and the thickness of the tendon. The larger the thickness the greater the load it could be bear. The present results propose an interesting fact about the mechanical properties of the SDFT and DDFT in donkeys. Thus, determination of the in vivo tendon biomechanics is very important; because overload of a tendon is best expressed in term of overstrain which could be clinically avoided by understanding these parameters **(Cohen et al., 1997; and Kane and Firth, 2008)**.

In this study, we used adult donkeys at age five years as a demonstration for measurement of mature digital flexor tendons biomechanical properties. This was in agreement with **Cherdchutham et al. (2001)** who found that, by 11 months of age the in vitro biomechanical properties of foal SDFT approximate those of adult.

The influence of strain on fore- and hindlimb in the present study, showed a significant increase in the SDFT and DDFT in the hindlimb. This reflects an extreme difference between the forelimbs and hindlimbs in equidae. This result is in agreement with **Holmstrom et al. (1994) and Back et al. (1995)** who reported that the hind limbs play a major role in equine locomotion.

CONCLUSION

In conclusion, MRI, ultrasonographic and biomechanical evaluation of the normal digital flexor tendons in donkeys provide a useful information about the normal anatomical features of these structures help in understanding of tendon injury and/or repair.

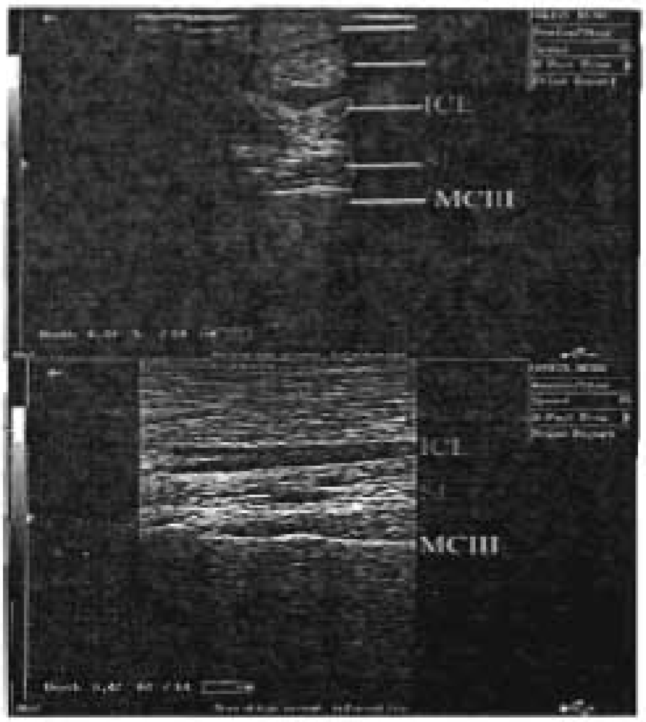

Fig (1): Transverse (A) and longitudinal (B) sonogram of the normal flexor tendons at the proximal metacarpal/metatarsal region showing the normal echogenicity and fiber alignment of the SDFT, DDFT, ICL and SL.

Fig (2): Transverse (A) and longitudinal (B) sonogram of the normal flexor tendons at the middle metacarpal/metatarsal region showing the normal echogenicity and fiber alignment of the SDFT, DDFT, ICL and SL.

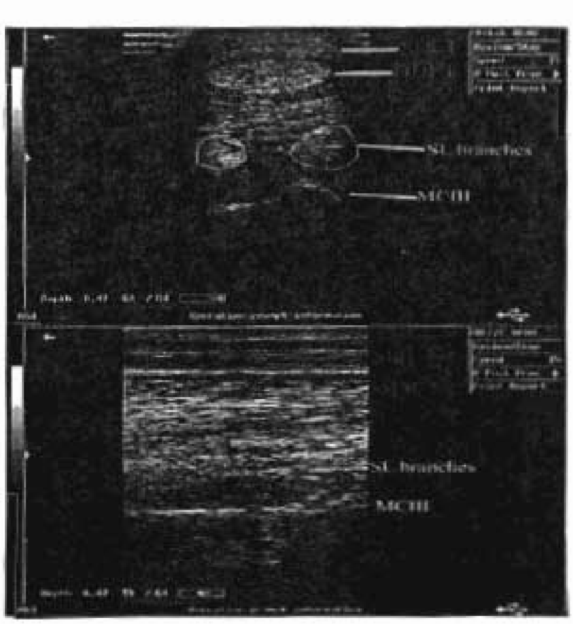

Fig (3): Transverse (A) and longitudinal (B) sonogram of the normal flexor tendons in the distal metacarpal/metatarsal region showing the normal echogenicity and fiber alignment of the SDFT, DDFT, ICL and SL branches.

Fig (4): Transverse (A) and longitudinal (B) sonogram of the normal flexor tendons at the level of fetlock joint showing the normal echogenicity and fiber alignment of the SDFT, DDFT, intersesamodin ligament and splint bone margin.

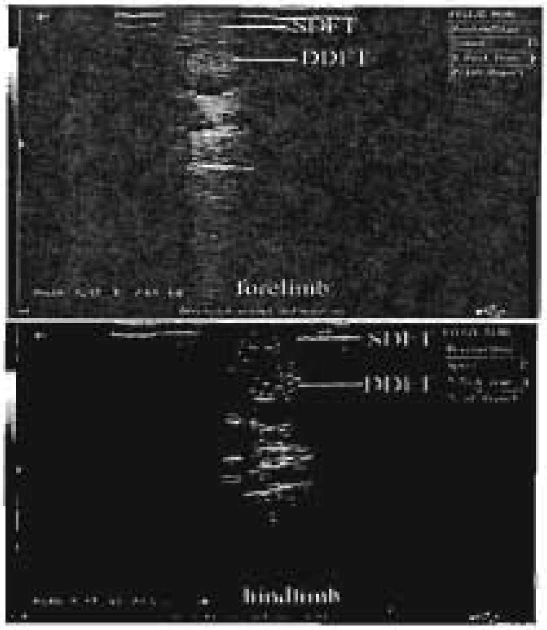

Fig (5): longitudinal sonogram of the normal flexor tendons in the proximal metacarpal region (A) and metatarsal region (B) showing the relation between the SDFT and DDFT in the fore and hind limbs.

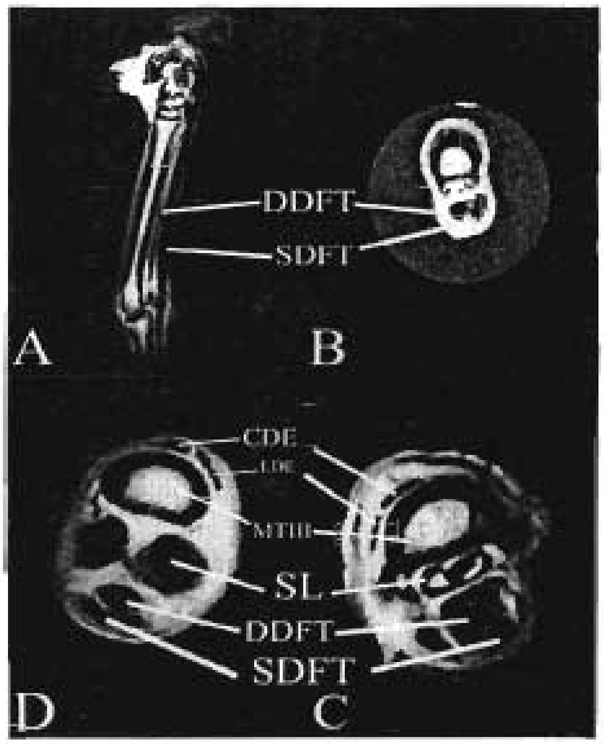

Fig (6): Normal MRI slice planes of sagittal (A) and transverse images of the metacarpal/metatarsal region showing the normal digital flexor tendons at the three divisions of the metatarsal region (B, C& D).

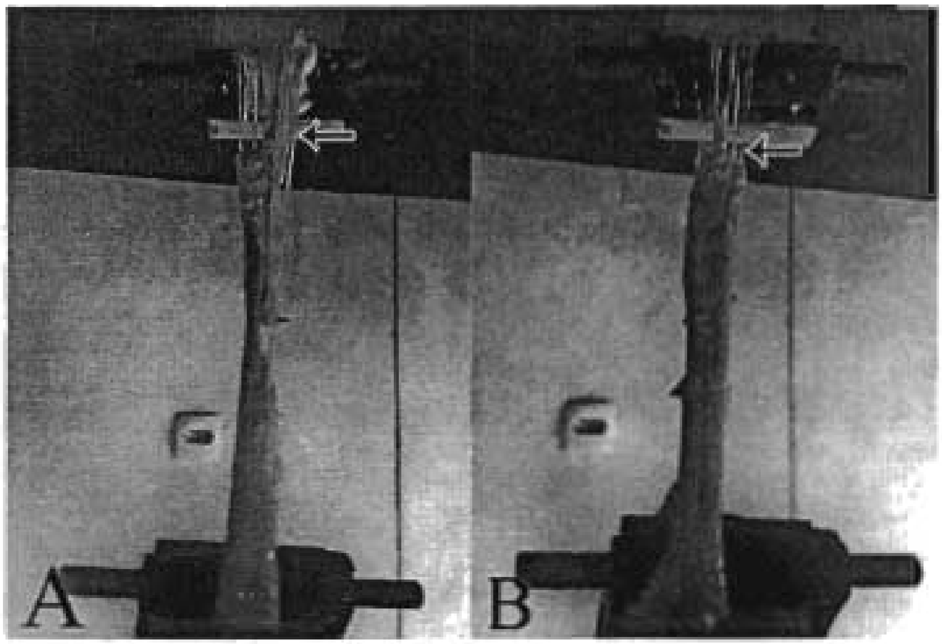

Fig (7): Failure of the normal specimen of SDFT (A) and DDFT (B) at the point of specimen gripping to the LLOYD arm.

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الملخص العربى

استخدام الرنين المغناطيسي والموجات فوق الصوتية والآلية الحيوية لتقييم أوتار القدم القابضة في الحمير

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صممت هذه الدراسة التجريبية لعمل تقييم مقارن لأوتار القدم القابضة في الحمير بواسطة الرنين المغناطيسي والموجات فوق الصوتية والآلية الحيوية ٠وقد أجريت هذه الدراسة على خمسة من الحمير وذلك لفحص أوتارها باستخدام الموجات فوق الصوتية، وقد تم إعدام هذه الحمير واستخدام أقدامها لعمل رنين مغناطيسي واختبار الخواص الآلية الحيوية لأوتارها ٠أظهر الفحص بالموجات فوق الصوتية لأوتار القدم القابضة السطحية والغائرة وجود اختلاف في الشكل واللون بينهما خاصة في الجزء العلوي منها، كما ظهرت هذه الأوتار في شكلها الطبيعي باللون الأسمر في صورة الرنين المغناطيسي، بينما أوضح قياس الخواص الآلية الحيوية فروق واضحة في قوة الشد بين الأوتار السطحية والغائرة في الأقدام الأمامية والخلفية - أثبتت نتائج هذه الدراسة أن استخدام الرنين المغناطيسي والموجات فوق الصوتية والآلية الحيوية لتقييم أوتار القدم القابضة يوفر معلومات مهمة عن الصورة التشريحية لهذه الأوتار مما يساعد على فهم إصابات الأوتار وطرق التئامها.