RELATIONSHIP BETWEEN NORMAL APPEARANCE OF FIBRE-LIKE STRUCTURE AND DEGENERATIVE CHANGES IN EQUINE DEEP DIGITAL FLEXOR TENDON

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ABSTRACT. Tendon in horse is the most important type of connective tissue which connects muscle to bone, constituting a vital component of the musculoskeletal system, by enabling movement. Tendons suffer from a wide range of disorders, which includes different types of mechanical injuries and degenerative diseases.

The sample population was a deep digital flexure tendon (DDFT) at mid-meta carpal region of thirty adult horses examined using scanning electron microscopy (SEM). Sex distribution was not taken into account. Changes in its structural organisation due to aging following tendon degeneration was unknown. The complex structures of tendon and its distinctive characteristics have been well demonstrated by SEM techniques. The tendon structure under SEM showed a dense, regular connective tissue arrangement that reflects the mechanical requirement of this tissue. It is defined by thick regular bundles of longitudinal collagen fibres arranged in a zig-zag conformation. All tendons examined from these horses had no history of deep digital flexure tendinitis and were all macroscopically normal. The tendon showed a hierarchical structure, with collagen molecules forming fibrils (50 nm to 500 nm), which in turn comprise a fascicle (50 µm to 300 µm), and fascicles were aligned along the longitudinal axis of the tendon.

Keywords: horses, DDFT, degeneration, normal fibre, SEM

INTRODUCTION

Tendons connect muscle to bone and enable forces generated by the muscle to transmit to bone causing movement of the skeleton. Flexor tendons flex the digit by two phases, the swing phase and the stance phase. They are loaded under tensile stress up to two times body weight (Reid N. and Besley J.E., 1991). During the stance phase, flexor tendons store and release elastic (Goodship *et al.,* 1994); The collagen fibres in tendon determine its material properties, giving it tensile strength and reducing stress exerted during muscle contraction (Kvist M. *et al.*, 1991). This structure consists of highly specialised connective tissue which is characterised by hierarchically arranged filaments embedded in a hydrophilic matrix with a relatively small volume of cells. The extracellular matrix (ECM) consists of collagen, elastin, proteoglycans and glycoproteins (Goodship *et al.,* 1994);

The biomechanical parameters of the equine digital flexor tendons involves superficial digital flexor tendon (SDFT), DDFT, suspensory ligament (SL) and distal accessory ligament conjugation, ultimate tensile stress, ultimate tensile strain and elasticity. The mechanical factors are important in causing tendon and ligament lesion. The distribution of overloads of several tendons in the equine limb has been studied extensively *in vitro* and *in vivo* (James R. *et al.*, 2008; Vergari C. *et al.,* 2011; Barrett E. *et al.*, 2014). SDFT forms a crescent shape when viewed transversely, with a concave dorsal surface and convex palmar/plantar surface, while DDFT cross-sectional shape is more rounded (Dyson S. *et al.*, 2007; Zubrod C. *et al.,* 2007).

The tension of the DDF apparatus contributes passively to initiate flexion of the carpal, metacarpophalangeal and interphalangeal. Injuries at the mid-metacarpal tensional region of the positional DDFT and the common digital extensor tendon (CDET) are rare. Injuries to the DDFT have been reported (Murray R.C. *et al.*, 2006) at both the phalangeal level (Smith M.R. and Wright I.M., 2006) and within the digital sheath. Tendon injury is due to overloading and strain (Clegg P.D., 2012; Dahlgren L.A., 2007; Thorpe C.T. *et al.*, 2010).

The aim of this study was to examine the structure of the DDFT at the mid-metacarpal region of clinically normal horses using SEM, and to study the comparative relationship between a normal DDFT and a degenerative tendon .

MATERIAL AND METHOD

Tendon samples

Equine DDFT was used in this study. Samples were collected from the forelimbs of thirty adults horses of different ages (15 adults, 13 middle-aged and 2 old age) at the mid-metacarpal region using SEM (Table 1). All horses in this study were evaluated to ensure that they were clinically normal without any signs of injury or lameness.

Scanning Electron Microscope (SEM) Techniques

Fresh samples of the DDFT from the left limb were isolated from young, middle-aged and old horses at the level of the mid-metacarpal region. The tendon specimens were collected by cutting the tissue into 1 cm cubes and preserved in Karnovsky's fixative for 12 to 24 hours at 4 °C. The samples were

TABLE 1: The number of horses and their ages using in this study.

Ages of Horses	Number of horses
8-10	15
12-17	13
18-25	2
Total	30

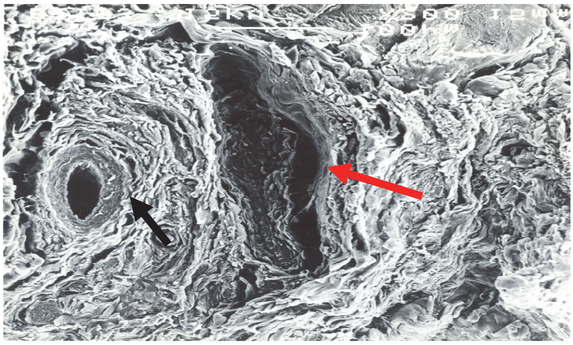

Figure:1 Transverse section of the DDFT showing the internal blood supply of the tendon red arrow is vein and the black arrow is artery. Bar = 100 µm

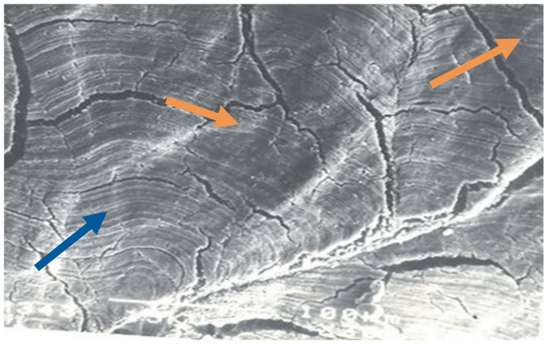

Figure 2. DDFT showing the area of tendon degeneration (brown arrows) compared with normal regular bundle of collagen fibres arranged in wave pattern of zig-zag conformation (blue arrow) under SEM. Bar = 100 µm

then washed with 3 changes of 0.1M sodium cacodylate buffers for 10 minutes each. The samples were post fixed in 1% buffered osmium tetraoxide for two hours at 4 °C again washed with another 3 changes 0.1M sodium cacodylate for 10 minutes each. The samples were dehydrated in a series of acetone of different concentrations (35%, 50%, 75% and 95%) for 10 minutes each and lastly washed in 3 changes absolute acetone for 15 minutes each (Hayat M.A., 1970; Reid N. and Besley J.E., 1991; Flegier S.L. *et al.*, 1993). The samples were then transferred into specimen baskets, put into critical point drying for approximately 180 minutes and mounted onto the stub using double-sided tape. Lastly the specimens were gold coated in the sputter and examined under scanning electron microscope (Joel, Japan).

RESULTS AND DISCUSSION

Overall study population

The 30 cases for inclusion to the study population were predominantly racing horses. For the overall study population the most commonly affected structure was the DDFT at the cross-section area (CSA).

The morphology of DDFT incidence of degenerating lesions of 15 adult horses aged 8-10 years old was 50%, 13 middle-aged horses aged 12-17 years old was 43.3% and 2 old horses aged 20-28 years old was 6.7%. As a result of all these physiologically age-related changes, an aged tendon is weaker with a likelihood to tear or suffer injury from overuse.

All of these tendon samples was observed under SEM which showed that the small and large bundles were of different lengths and surrounded by a reticulum of connective tissue. The wave pattern of the DDFT appeared as zigzag formation on the surface under the SEM (Figure 2). The longitudinal section revealed that the tendon received much of its blood supply through branches of the median artery and vein (Figure 1). The changes due to degeneration at the mid-metacarpal region of the DDFT appeared as black homogenous areas indicating a loss of the uniform wave pattern that characterised a normal healthy equine DDFT. The results of this study suggest that SEM could play an important role in investigating normal dynamic tendon structure. Tendon degeneration is a complex process and is the result of several interacting factors.

In general, degeneration leads to a decrease in function from the cellular level to the organ level (Buckwalter J.A. *et al.*, 1993). Although the exact mechanism of tendon degeneration is not properly understood, it was suggested that the mechanism of tendon degeneration is due to hyperthermia of the tendon (Wilson A.M. and Goodship W.E., 1994). Several other theories have been forwarded including passive mechanical degeneration, active degradation through the action of vascular reduction and neural overstimulation. These degenerative changes have been studied (Asheim A., 1964; Adams O.R., 1974; Stromberg B., 1971). Overstress of tendon may also lead to injury and increases with increasing age (Ely E.R. *et al.*, 2009; Ely E.R. *et al.*, 2004; Kasashima Y. *et al.,* 2004; Perkins N.R. *et al.,* 2005). The results of this study were similar to their findings.

Degenerative change have been reported in the homologous tendon in humans (Asvazadurian A.O. and Marini C., 1961) where the most common site of injury is between the central carpal and fetlock sheath. This is similar to the results of this study. This area is particularly poorly vascularised compared to the rest of the tendon. As a consequence of prolonged exercise and restriction of blood supply, ischemia may develop which lead to tendon damage. These observations are similar to those in thoroughbreds (Miels C.A. *et al.*, 1994) and standardbred (Nixon A.J., 1990).

Tendons have a lower oxygen consumption compared to other tissues allowing tendons to function for longer periods of time without fatigue (Vailas A.C. *et al.,* 1978). Some research had suggested that the raising of a horse's heel will decrease the load on the DDFT, increasing fetlock joint extension and subsequently the load on SDFT and SL (Crevier-Denoix N. *et al.,* 2001).

At this moment no references have been found to describe the degenerative change in the DDFT at the level of CSA.

CONCLUSION

In conclusion, the study showed that the structure of the normal equine DDFT can be clearly assessed using SEM. This technique can be an useful instrument for the characterisation of thick tendon sections. It is anticipated that the technique can be a useful tool in the diagnosis of tendon injury.

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