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Age Related Histological Changes of Flexor Tendons of Forelimb in the Buffalo Bull *(Bubalus bubalis)*

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Abstract: ***Animal gait is dependent mainly on its limb musculature, ligaments and tendons which are appreciable superficially. Body conformation of Bovines and their management practices in India make these anatomical structures prone to injuries. In the present study, post-natal age-related microscopic features of Superficial Digital Flexor Tendon (SDFT)and Deep Digital Flexor Tendon (DDFT) of the forelimb of buffalo bull were conducted. Chondrocyte-like cells were noticed at myo-tendinous junction which increased in number with advancing age from GI (1-3 yrs) to G3 (> 6 yrs). At myo-tendinous insertions isolated clusters of chondrocytes like cells along with scattered cells and adipose tissue were seen in G II (3-6 yrs) and GIII. Collagen crimps reduced with advancing age. DDFT had short stumpy muscle fibers oriented in different directions at myotendinous junction. At mid metacarpus the collagen crimps were noticed which decreased with the increasing age. Adipocytes were seen along connective tissue sheaths surrounding the tendon from mid metacarpal level to insertion point in both tendons.***

Keywords: collagen crimps, chondrocyte, flexors, fore limb, bovines

1. Introduction

Outer most covering of a tendon is loose connective tissue, the paratenon under which a connective tissue sheath, epitenon encloses the whole tendon. It continues externally with paratenon and internally with endotenon, a thin reticular network of connective tissue which carries blood vessels, nerves and lymphatics to the deeper parts of the tendon (Kannus, 2000).

As age advances, biochemical, cellular, mechanical and pathological changes are noticed in a mature tendon, which results in structural changes and decreased functioning of a tendon (Tuite, 1997).

2. Materials and methods

The present investigation was carried out in Department of Veterinary Anatomy, College of Veterinary Science, Hyderabad. Fore limb specimens of 36 apparently healthy buffalo bulls of different post-natal ages were procured from local slaughter house in Hyderabad. Forelimb specimens with intact skin were collected from the level of carpus up to the distal phalanx including hoof. Aging of post-natal specimens was done by noting down the dentition pattern of slaughtered animals according to FAO (1994) *viz.*, 1 to 3 yrs (group I), 3 to 6 yrs (group II) and 6 yrs and above (group III).

The tissue samples from SDFT and DDFT were collected at three different sites *viz*, at their origin, mid-metacarpal course and insertion points and were immediately fixed in 10 % Neutral Buffered Formalin (NBF)and processed for routine paraffin technique. Microscopic sections of 5 – 6 µm were cut and subjected to the routine H & E staining technique, Masson's trichrome and Verhoeff's stain as per Singh and Sulochana (1997).

For histochemical studies paraffin sections were stained with Periodic Acid Schiff technique (PAS), PAS-Alcian Blue method and fresh unfixed sections were stained with Oil Red 'O' technique (Singh and Sulochana, 1997).

3. Results and discussion

In the present study both SDFT and DDFT at myo-tendinous junction comprised wavy bundles of collagen intermingled with muscle fibres oriented in different directions. At carpal level the tendons were covered by moderately thick epitenon below which were numerous collagen bundles demarcated by paratenon, a continuation of epitenon was seen in aged specimens in both digital flexors. In groups II and III elaborate distribution of connective tissue in DDFT clearly defined the collagen bundles. Blood vessels of different sizes were noticed in all regions of epitenon, para and mesotenon in both tendons (Fig. 1).

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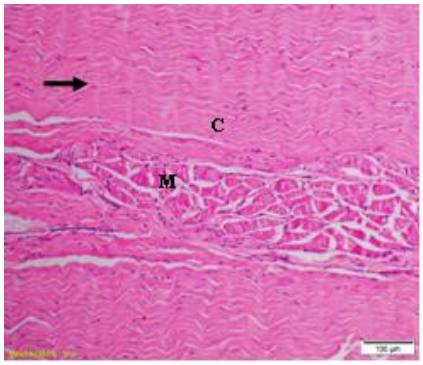

Figure 1: Longitudinal section of DDFT at mid metacarpus showing the parallelly arranged collagen fibers (C) muscle bundles (M), among which tenocyte nuclei (→) can be seen in group I (H&E 4X)

These findings are similar with histology of mammalian tendons described by Eurell and Frappier (2006) in domestic mammals; Ham and Cormack (1987), Eroschenko (2008) and Gartner (2015) in human tendons. They stated that mammalian tendon was made up of numerous fascicles of parallelly arranged wavy collagen fibers, enveloped by fibrocyte-lined endotendinium. They noticed that peritendinium continued with epitendinium to cover the entire tendon and comprised many blood vessels.

The above authors opined that elaborate connective tissue arrangements with its constituents actually helped in putting up strong resistance against the forces that pulled the tendon in a single axis or direction. Similar vascularisation of human digital flexors was also expressed by Langberg *et al.* (1998), Kostopoulos*et al.* (2006) and Benjamin *et al.* (2008) stated that as a general rule, tendons have vascular supply less than that of associated metabolically active muscles because of which fresh tendons appear more paler than muscle. Contrary to the view of earlier anatomists, they mentioned that tendons are still vascularised which is important for their normal function and their ability to repair.

In equines Webbon (1978) described characteristic four regions *viz.*, musculo-tendinous junction (MTJ) and tendon within carpal sheath, metacarpal (extra-synovial) region, tendon within digital sheath and lastly insertion of the tendon. Similar regions were evident in histological sections of the SDFT and DDFT in buffalos in this study.

Webbon (1978) stated that SDFT in young horses had numerous, plump and elongated tendon cell nuclei, whereas in 5 yr-old the tendon consisted of intensely basophilic fine fibrillar areas. At metacarpal region the tendons had loosely arranged vascular tissue the 'paratenon' below which eosinophilic fibres were in undulating form whose amplitude decreased with age. He mentioned that tendon cell nuclei in 2 yr-old horses were elongated than neonates, but after 2 yrs of age cellularity reduced and with increasing age their number reduced and chondroid cells increased.

Similar features were noticed in SDFT and DDFT in this study where long and wavy collagen fibres were seen at mid metacarpal in all groups. Muscle fibres in the interior were seen as short and fragmented structures intermingled with collagen fibres (Fig. 2). Blood vessels in proximity of collagen bundles were noticed with numerous fibroblast-like cells along with isolated chondrocyte-like cells.

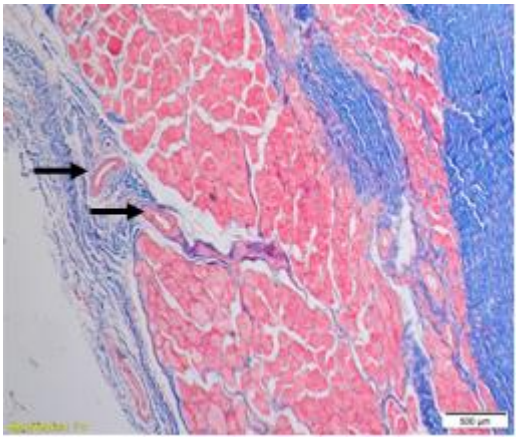

Figure 2: Transverse section of DDFT at its origin showing the muscle bundles (red) and collagen fibers (blue) with the vasculature (→) in epitenon in group III (Masson's Trichrome 2X)

In this study tenocyte nuclei were relatively longer and numerous in groups I and II than of that of older group tendons. From group II onwards adipocytes were also seen in mid metacarpal region (Fig. 3). The above features increased towards the insertion points in SDFT and DDFT across all groups, especially the presence of clusters and isolated chondrocyte-like cells were relatively more in groups II and III. Close to insertion of the tendon, collagen crimps were not appreciated.

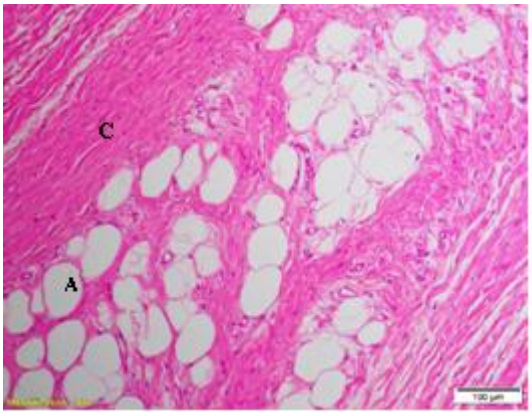

Figure 3: Longitudinal section of DDFT at insertion showing adipocytes (A) among the parallelly arranged collagen bundles (C) in group III (H&E 10X)

Our findings correlate with the description of flexor tendons by other authors in humans, ruminants, horses and other mammals (in horses by Webbon, 1978, Goodship, 1993 and Gillis, 1997; in humans by Hess *et al.*, 1989, Kannus, 2000 and Benjamin *et al.*, 2008 in humans; Gelberman *et al.*, 1984 in chicken, rabbit, dog and monkey and in bovines by Vogel and Peters, 2005 and Takahashi *et al.*, 2018). Vogel and Peters (2005) concluded that bovine deep flexor tendon had a rich network of blood vessels in the peritenon on its posterior surface as well as in the longitudinal core of tendon substance.

Histochemical features

In this study, PAS reaction was mild or negligible in collagen fibres in all groups of both SDFT and DDFT

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tendons. It was moderate in linings of blood vessels, which populated connective tissue in all groups (Fig. 4).

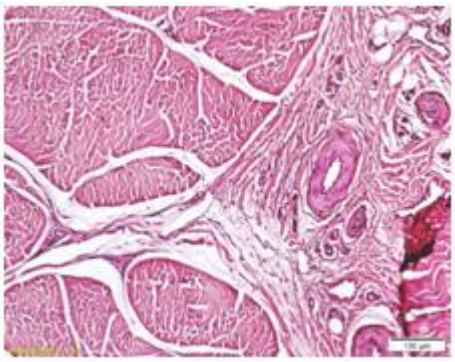

Figure 4: Transverse section of SDFT at mid metacarpus showing mild PAS reaction in internal elastic lamina of the blood vessel group III (PAS 20X)

Collagen fibres were inert to this reaction. In case of PAS-Ab reaction it was moderate in connective spaces in between collagen bundles in both tendons in all groups, but was strong in chondrocyte-like cells and surrounding vascular elements (Fig. 5). Our observations concur with the results of Vogel and Peters (2005) who stated that mid-substance fibrocartilage develops in bovine deep flexor tendon at the point where it wraps under sesamoid bones of foot. They said that this proteoglycan-rich tissue, which is subjected to mechanical loading other than pure tension stained intensely with Alcian blue along with surrounding vascular elements at a point where several fiber bundles come together. Positive reaction to PAS-Ab around blood vessels and chondrocyte-like cells in SDFT and DDFT of forelimbs in this study indicated the presence of proteoglycan. This feature is akin to reports of Vogel and Peters (2005), who stated that this may serve multiple roles such as providing compressive stiffness separate and lubricate collagen bundles that move relative to each other, but may also protect the integrity of vasculature in tendon subjected to bending and shear.

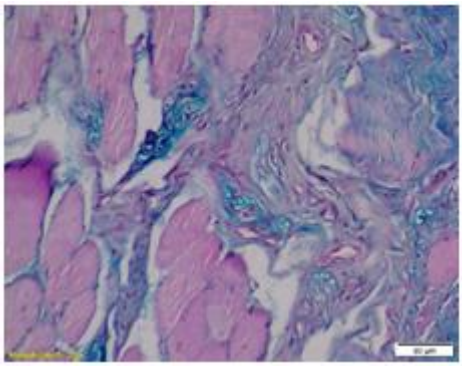

Figure 5: Transverse section of DDFT at origin showing mild reaction to PAS-Ab in blood vessel endothelium and moderate reaction in cytoplasm of chondrocyte-like cells in group III (PAS-Ab 20X)

Similar studies of tensile and compression regions of frog plantaris longus tendon was recorded by de Carvalho and de Campos Vidal (1994). They suggested existence of large proteoglycans due to intense Alcian blue reactivity in fibre bundles and associated substances in compression region of tendon. In this study, regions proximal and distal to inter-digital space of manus are the 'tension' and 'compression' zones of the digital flexor tendons.

Lipids were localised in adipose tissue of epitenon and mesotenon of both tendons in older groups at mid metacarpal to insertion regions in this study (Fig. 6). Such lipid accumulation in large tendons was reported by Adams *et al.* (1974) in human tissues, who stated that lipids in tendons were extra cellular, fine droplets along the axis of the collagen fibres in tendons and fascia in ageing humans. Lipid deposits occurred from 15 years onwards and were more significant beyond 45 yrs. They stated that large tendon like Achilles tendon showed severe lipid deposition.

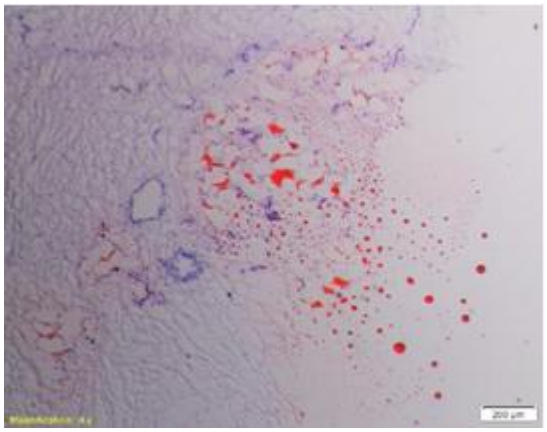

Figure 6: Transverse cryosection of SDFT at mid metacarpus of group II showing the reaction to lipids in the epitenon (Oil Red O 10X)

4. Conclusion

This investigation establishes that digital flexors of forelimbs in buffalo are designed not only to bear major portion of the body weight but also suited to withstand external forces and is sufficiently reinforced with extensive connective tissue studded with blood vessels of different sizes, all of which play an important role in tendon repair.

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