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Platelet Rich Plasma in the Treatment of Superficial Digital Flexor Tendon Core Lesion in Donkeys: Histopathological Assessment

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ABSTRACT

The aim of this study was to assess the effect of platelet rich plasma (PRP) on the healing of created core lesions in the superficial digital flexor tendon (SDFT) in donkeys. Serial histopathological specimens were taken from normal and at 5th, 15th, 30th, 60th and 90th days post PRP treatment. Histopathological findings showed early rapid healing process with early angiogenesis, increased fibroblastic condensation, the nuclei appeared more elongated, flattened arranged in the peripheral collagen fibers. The collagen fibers changed from randomly arranged to semi-parallel and finally highly reoriented parallel closely packed collagen fibers at 90th days of PRP treatment. Compared to saline control displayed few scattered fibroblasts and angiogenesis. As well as less regularly arranged collagen fibers. Histopathological evaluation 90 days after three successive PRP treatment proved a more rapid tendon tissue reconstruction and maturation similar to the healthy tendons.

Key words: Core lesion, Donkey, Histopathology, Platelet rich plasma, Tendon repair.

INTRODUCTION

Platelet-rich plasma (PRP) is a product derived from the whole blood that contains high concentrations of platelets that have chemotactic, neovascular, mitogenic and healing properties (Kwirant *et al.* 2019). The PRP has numerous cytokines and growth factors such as platelet derived growth factor, transforming growth factor-ß and vascular endothelial growth factor (Carmona *et al.* 2018 and Miranda *et al.* 2018). These factors perform specific actions during tendon healing and enhance tenocyte proliferation (Mazzocca *et al.* 2013), vascular density and collagen and matrix synthesis (Bosch *et al.* 2011). PRP is effective in reducing pain in damaged soft tissues (Textor and Tablin, 2013), improve biomechanical properties in healing SDFT lesions (Bosch *et al.* 2010) and increase the number of horses reaching their previous level of performance (Geburek *et al.* 2016 and Alzola *et al.* 2018).

The superficial digital flexor tendinopathy represented 11- 30% of musculoskeletal lesions in Thoroughbred racehorses (Brandão *et al.* 2018).Tendons have a low ability to heal (Kaneps, 2016). Several treatments have been tried including the local or systemic application of anti-inflammatory drugs, intratendinous injections with corticosteroids (Dyson, 2004), PRP (Bosch *et al.* 2010 and Geburek *et al.* 2016) and stem cells (Geburek *et al.* 2017).

Previous studies proved that PRP treatment had significant positive results leading to better organization of collagen fibrils, less inflammation and increased vascularity in tendinopathy in the horses (Boch *et al.* 2010; Gebruek *et al.* 2017 and Alzole *et al.* 2018). There are scarce studies on PRP treatment of tendon injuries in donkeys, particularly, systematic histopathological investigation in donkeys after PRP treatment. Therefore, the aim of this study was to assess the effect of PRP treatment on the histopathological findings during different stages of healing process on surgically created SDFT core lesions in donkeys.

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MATERIALS AND METHODS

Animals

Fourteen clinically healthy donkeys free of lameness aged 3 to 9 years (4.6±0.3) and body weight 115 to 160 kg (127.66±2.2 kg) were included in this study. Twelve clinically healthy donkeys were used for creation of SDFTs core lesions and the other two were used for normal SDFTs histopathology. This research work had been approved by the Benha University committee on the care and use of experimental animals (BNFVTM 01-04-2019).

Surgical creation of core lesions and PRP preparation

Core lesions were created, surgically under ultrasonographic guidance, in the mid palmar region of SDFTs of both the

fore limbs just proximal to the digital sheath as described by Schramme *et al.* (2010). After aseptic preparation a small skin incision in the palmar midline. A longitudinal tunnel was created using 2.5-mm blunt conical obturator (Karl Storz, Germany) was inserted and guided inside the tendon core under ultrasonographic guidance for a distance of 10 cm. followed by a 2.5-mm burr (Abradar burr, Karl Storz) was inserted into the tunnel, activated and gradually pulled backward over 15 seconds. Epitenon incisions were sutured and the skin was suture with simple interrupted suture patterns. Bandaged were applied over the wounds until intralesional injections. All donkeys received Xylazine, (Xylaject, Adwia Co., Cairo, Egypt.) @ 1.0 mg/kg IV and Thiopental Sodium (Thiopental Na, Novartis Pharma-Egypt) @ 6-8 mg/kg IV as described by Hall *et al.* (2001).

Preparation and Injection of Platelet Rich Plasma (PRP)

The PRP was prepared according to the technique described by Bosch (2009).The mean platelet count in the whole blood was 148.17±15.8×10^6 platelets /ml and the mean platelet count in the PRP samples were 437 ± 31.8×10^6 platelets/ ml. The PRP was activated by addition of calcium chloride 10% (0.1 ml calcium chloride to 1ml PRP) prior to injection as described earlier (Zuffova *et al.*2013). Five days post surgery and under ultrasonographic guidance, one randomly front limb core lesion was treated with 3-4 ml of PRP at 5^{th},15^{th} and 30^{th} days, the contralateral limb was saline treated as control at the same time in twelve clinically healthy donkeys.

Euthanasia and histopathology specimens

At 5^{th}, 15^{th}, 30^{th}, 60^{th} and 90^{th} days after the creation of SDFTs core lesions in treated and control groups were humanely euthanized using an overdose of pentobarbital @ 200 mg/ kg BW, IV (Euthesate, CevaSanteAnimale) after sedation with Xylazine @ 1 mg/ kg bwt I/V. Autopsy samples were taken from the normal SDFTs and core lesions donkeys and fixed in 10% formol saline for 24 hours. Longitudinal 5-µm thin sections were cut and stained using hematoxylin and eosin for evaluation of changes in normal tendon architecture; degeneration, hemorrhage; fibroblast shape; angiogenesis and organization of collagen fibers, proliferative phase and the maturation phase was evaluated subjectively as described by (Zuffova *et al*. 2013).

RESULTS AND DISCUSSION

The aim of this study was to assess the efficacy of PRP in treating surgically induced core lesions in SDFT in donkeys based on histological findings. The mean platelet count in the PRP in the current study was 437.0 ± 31.8×10^6 platelets / ml which represented 3.4 ± 0.4 folds more platelets in PRP as compared to whole blood which corroborated with previous studies on horses (Arguelles *et al*. 2008; Gebruek *et al*., 2016; Maia *et al*., 2018). The high concentration of PRP have been reported to result in high amount of released growth factors and subsequent high biological effects (Alozla *et al*., 2018). Similar to as described in the horse (Gebruek *et al*. 2016; Gebruek *et al*. 2017), the central core lesions in the SDFT of donkeys were successfully induced, surgically under the guidance of ultrasound, in the current study.

The normal histology of the SDFT in donkey in the present study appeared closely packed, highly organized and parallel thick collagen bundles (Fig1A). The fibroblasts showed the linear shape and flattened nuclei arranged in the periphery of collagen bundles. The same findings were described by Zhang *et al.* (2013).

The created core lesions 5^{th} days after surgery were an absence of normal tendon architecture with marked disorganization of collagen fibers, massive inflammatory cells infiltration and focal areas of hemorrhage (Fig 1B). Similar findings have been demonstrated in tendinopathy in the horse by Tsukiyama *et al.* (1996); Schramme *et al.* (2010) and Gebruek *et al.* (2017). Moreover, Alozla *et al.* (2018) attributed the ultrasonographic anechoic appearance of SDFT lesions to hemorrhages, edema and early granulation tissue formation.

Bosch *et al.* (2011) and Gebruek *et al.* (2016) reported that the fibroproliferative phase of tendon repair started between 3^{rd} and 16^{th} days and reached its peak up to 4 weeks after PRP treatment. Moreover, the beginning of fibrovascular callus formation and collagen deposition

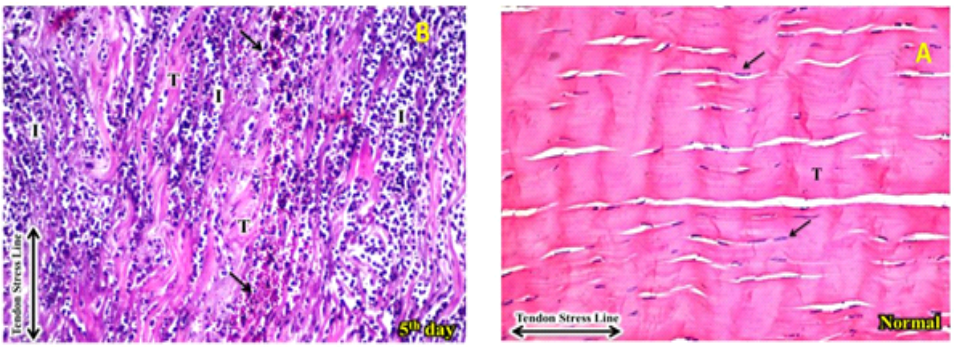

Fig 1A & B: Histopathological in saline control (A) and 5^{th} days post surgically created SDFT core lesion (B) in donkeys. The SDFT (A) closely packed collagen fibres (T). The Fibroblast linear flattened niclei (arrow). Core lesion (B) disorganized collagen fibres (T). Massive inflammatory cells (I). Focal areas of hemorrhages (arrow). H & E stain (X40).

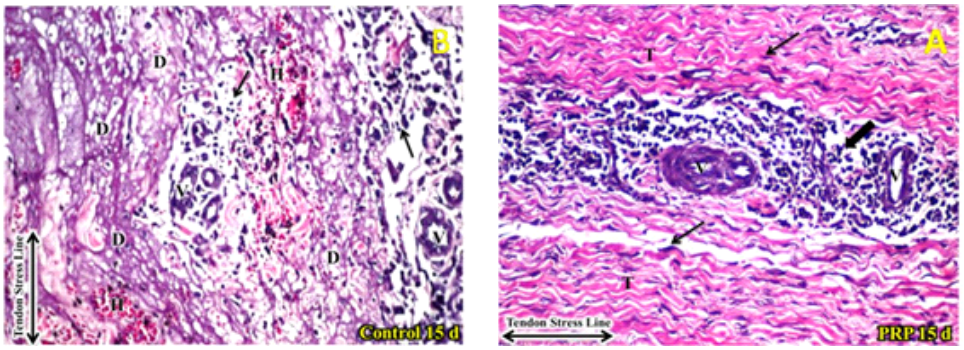

Fig 2A & B: Histopathology of surgically created core lesions 15th days after treatment with PRP (A) and saline control (B). Early extensive neovascularization (V) and fibroblastic infiltration (Thin arrow). Inflammatory cells infiltration (Thick arrow). Collagen fibres intact (T). H&E stain (X 40).

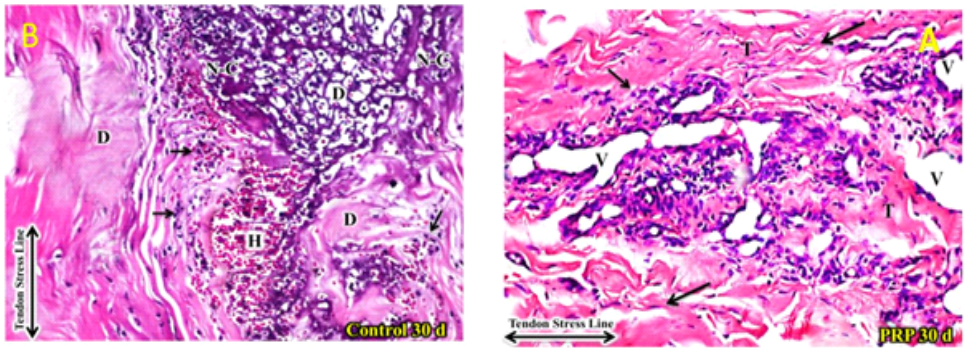

Fig 3A & B: Histopathology of surgically created core lesions 30th days after treatment with PRP (A) and saline control (B). Multiple numbers of large blood vessels (V). With Endothelial wall lining and irregular condensation of fibroblast (arrow). H&E stain (X40). Absense of normal tendon architecture (B). Severe hyaline and myxomatous degeneration (D). H&E stain (X40).

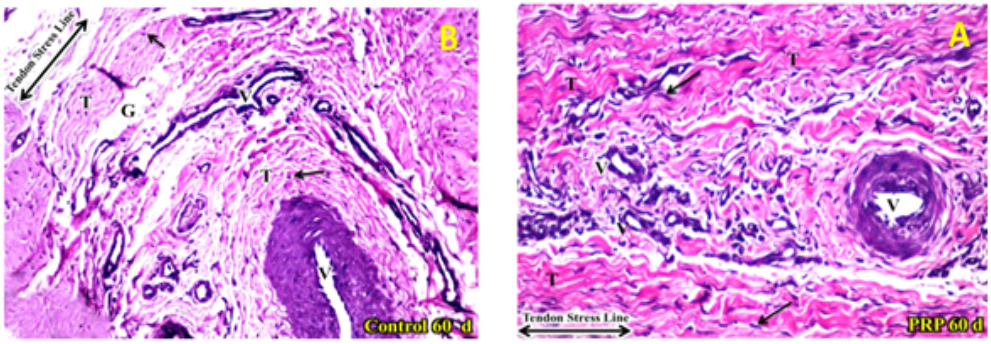

Fig 4A & B: Histopathology of surgically created core lesions 60th days after treatment with PRP (A) and saline control (B). The fibroblasts appeared elongated shape, flattened Nuclei (arrow). Collagen fibres appeard reoriented (T). Weak response of granulation tissue (B). Newly formated blood capillaries (V) and few scattered round fibroblasts (arrow). H&E stain (X40).

represented the initiated response of tissue regeneration and resolution of inflammatory reaction. These results corroborated with the findings during the 15th and 30th days after surgery in donkeys and represented the proliferative phase of tendon repair with increased angiogenesis, condensation of fibroblast and semi-regular collagen fiber thickens (Figs 2 A and 3A). Comparison with saline control had multiple focal areas of hemorrhage, necrosis and few scattered fibroblasts (Figs 2B and 3B) at the same time. The same observations have been reported in PRP treated horses (Mai *et al.* 2009). In addition, the observed angiogenesis during the proliferation and remodeling phase of tendon healing, in the current study, could be attributed to Vascular Endothelial Growth Factor (Molloy *et al.* 2003).

The repair process of PRP treated core lesions during 60th and 90th days after surgery displayed a reduced degree of fibrovascular response associated with the reconstruction of normal tendon structure. The fibroblasts appeared elongated in shape, flattened nuclei and arranged on the periphery of the collagen bundles. The collagen fibers appeared closely packed as thick bundles, highly reoriented, parallel to each other and extended to invade the remnant

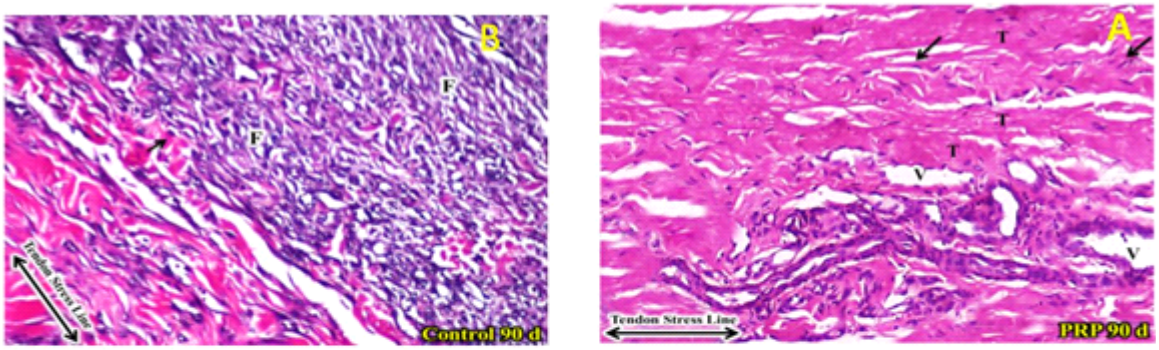

Fig 5A & B: Histopathology of surgically created core lesions 90th days after treatment with PRP (A) and saline control (B). Normal fibroblasts (arrow). Closely packed, reoreinted and parallel collgen fibres (T) similar to normal architectures. In (B) Diffused area of fibrosis (F) Round to ovoid fibroblast (arrow). Randomly arranged collagen fibres (T) at the end of study. H and H stain (X40).

of fibrovascular callus (Figs 4 A and 5A). However, saline treated tendons showed weak granulation tissue formation, absence of normal tendon architecture and extensive infiltration with round to ovoid fibroblast (Figs 4B and 5B).

Previous assessment of intralesional PRP treatment in the horses with tendinopathy showed early improvement of ultrasonographic features, reduction of lameness, advanced collagen fiber organization and successful return of horses to racing have been reported (Gebruek *et al.,* 2016; Alzola *et al.,* 2018). Similar observations have also been documented previously in donkeys after SDFT core lesions during PRP treatment (Mostafa *et al.* 2015a and 2015b).

Therefore, the obtained histopathological results after PRP treatment in surgically created core lesions in SDFTs in donkey showed a satisfying outcome of the healing process confirmed by excessive angiogenesis, condensation of fibroblasts with more elongated, flattened nuclei and highly reoriented parallel collagen bundles similar to the healthy tendons after 90 days from treatment.

CONCLUSION

Intratendinou PRP treatment has a significant early histopathological property of the repair tissue in surgically induced core lesions in donkey SDFT compared to saline control group.

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