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Autologous mesenchymal stem cells for treatment of acute superficial digital flexor tendonitis in athletic horses – a clinical study of 15 cases

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Summary: Acute tendonitis is typically associated with core lesion formation with accompanied degenerative changes and microdamage of collagen fiber cross-linking and regenerative therapy using mesenchymal stem cells (MSC) is one of the most advanced medical treatments. The aim of the present study is to investigate the therapeutic effect of autologous mesenchymal stem cells (MSCs) on athletic horses affected with acute superficial digital flexor tendonitis. A total of 15 mixed breed athletic horses confirmed to have acute tendonitis of SDFT were included. The affected SDFTs were confirmed via clinical examination and ultrasonography. Bone marrow was then harvested from the sternum of each horse using Jamshidi biopsy needle for MSCs isolation and culture. Auto implantation of a single dose of MSCs (1 ml, 10×10^6 cells) into the core lesion of each affected tendon was performed under US guidance after thorough aseptic preparation. After 2 weeks of stall rest, the horses were subjected to a rehabilitation program by daily hand walk for 15–30 minutes until the end of the follow-up period (8 weeks). All horses were subjected to clinical and ultrasonographic evaluation at 2, 4, and 8 weeks post injection. Ultrasonographic examination of the affected zones was carried out at the palmaro-metacarpal region to determine the length of the maximum injured zone lesion (MIZ%), the echogenicity of the core lesion, and the tendon fiber alignment pattern. Clinical and ultrasonographic findings were satisfactory after 8 weeks of MSCs therapy. In conclusion autogenous MSCs therapy has proven to have a reparative effect on acute tendonitis since the majority of treated patients (66.7%) have returned to athletic activity.

Keywords: horse, acut, tendonitis, MSCs, ultrasonography, stem cells

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Introduction

Acute tendonitis is typically associated with core lesion formation with accompanied degenerative changes and microdamage of collagen fiber cross-linking (*Reed* and *Leahy* 2013). These injuries result in lameness and require prolonged recovery time, and at the same time, carry a high risk of recurrence. The relatively prolonged lameness associated with tendon and ligament injuries results in major wastage of the horse performance, as well as the owner's time and money (*Gillis* et al. 1997). Superficial digital flexor tendon injury is a common cause of wastage among competitive horses with associated failure to return to the previous level of performance and high incidence of recurrence. Tendon lesions range in severity from mild inflammatory changes and minor partial unilateral tear to complete bilateral tendon rupture (*Goodship* 1993). Conventional treatments for tendonitis basically rely on clinical and surgical approaches. However, the recovered animals are rarely sufficiently healed to allow a full return to limb function with a performance similar to that observed when they were healthy (*Marfe* et al. 2012). Regenerative therapy using mesenchymal stem cells (MSC) is one of the most advanced medical treatments of tendon and ligament injury to restore normal architecture and biomechanical function of the affected tissue (*Brehm* 2006, *Barreira* et al. 2008, *Smith* 2010, *Geburek* and *Stadler* 2011, *Kasashima* et al. 2011, *Tummers* et al. 2012). Previous studies have shown a significant decrease in the recurrence rate of tendonitis following MSC therapy compared to conventional therapies (*Godwin* et al. 2012).

The present study therefore investigates the clinical and ultrasonographic outcomes after intra-lesional implantation of autologous MSC in athletic horses confirmed to have acute superficial digital flexor tendonitis. Our hypothesis is that horses treated with MSC are expected to rapidly return to full sports as a result of the associated potential repair of the core lesion of the injured tendons.

Materials and Methods

A total of 15 adult athletic horses of mixed breeds (8 mares and 7 male) aged 4 to 12 years with a weight of 350 to 450 kg, belonging to the Police Academy Equestrian Clinic in Cairo with confirmed unilateral superficial digital flexor tendonitis were selected for inclusion in the present study over the past two years (2017–2018). The study was approved by the Cairo University Institutional Animal Care and Use Committee (CU-IACUC, CU/II/F/27/2017). The age, body weight, sex, and breed were recorded and clinical and ultrasonographic examinations were performed to all enrolled clinically affected horses over the period of the study. The riders' complaint, horse's temperament, type of sport, condition of shoeing, and history of onset and duration of the disease were recorded. The clinically affected SDFT with tendonitis was confirmed via clinical examination and ultrasonography. Horses with SDF tendonitis along with abnormal limb conformation or other orthopedic problems were excluded from the study. The initial degree of lameness, signs of tendon reaction, flexion test response, ultrasonographic findings, and long-term clinical follow-up were recorded for each affected horse before and after treatment. Ultrasonogrphic evaluation of the affected tendons was performed and documented at the affected zones of the palmaro-metacarpal region transversally and longitudinally to determine the percentage of the maximum injured zone (MIZ %), the lesion echogenicity, and associated tendon fiber alignment pattern.

Bone marrow was harvested from the sternum using Jamshidi biopsy needle (Medax medical device, Poggio Rusco, Italy). For collection, each enrolled horse was sedated using an IV dose of xylazine 2 % (1.1 mg/kg) and secured in a stanchion. The sternum was palpated from the point between the fore limbs caudally to identify the three most caudal sternebrae based on the appearance of their intersternebral spaces. The location overlying the sternum was clipped, scrubbed, and subcutaneously infiltrated with 2 % lidocaine HCl. The sternum was then ultrasonographically examined to locate the 5th sternebra which was then marked with a pen. Under aseptic condition, a small stab incision was made through the skin and an 11-gauge, 10 cm biopsy needle was introduced through the stab incision (4–5 cm deep) until the tip of the needle made contact with the sternebra. Cautious pushing of the needle for a further 3 cm interiorly was performed to inter the bone marrow sinus and 10 ml of bone marrow were then collected using a syringe preloaded with 2 ml of heparin of (15000 IU/ml heparin, Global Pharmaceutical industry, Cairo, Egypt) (Fig. 1). The skin wound was then closed by one stitch. The bone marrow sample was then shipped to the laboratory in an ice box for mesenchymal stem cells (MSCs) isolation and culture. Mesenchymal stem cell (MSC) isolation and culture was performed according to (*Rickard* et al. 1996).

Fig. 1 Collection of sternal bone marrow by Jamshidi biopsy needle. Left sector (Jamshidi needle photo)

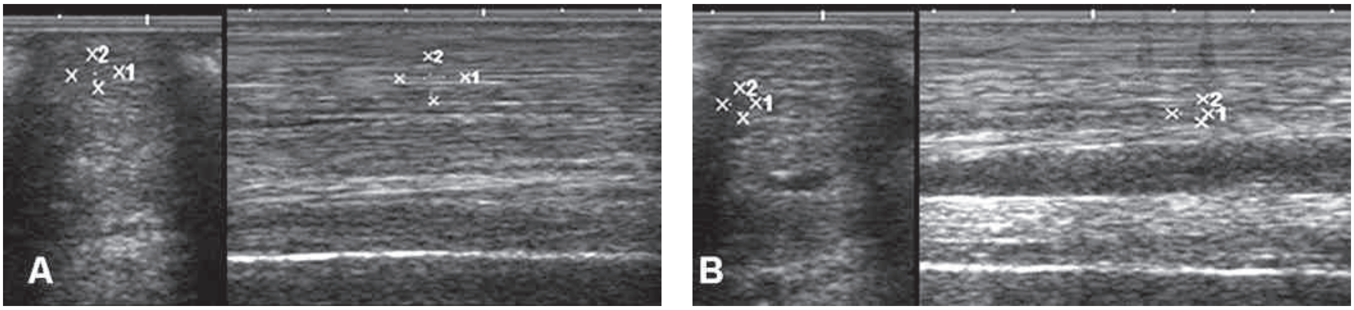
Table 1	Grades of lameness (modified from *Stashak* 2002).
0	Lameness not perceptible under all circumstances.
1 (Mild)	Lameness is difficult to observe and is not consistently apparent, regardless of circumstances.
2 (Moderate)	Lameness is consistently observable at a walk or a trot under all circumstances.
3 (Severe)	Minimal weight bearing lameness in motion and/or at rest or a complete inability to move.

A single intra-tendinous (intra-lesional) injection of 1 ml of autogenous bone marrow derived MSCs (10×10^6 MSCs) was performed to each clinically affected horse under US guidance and thorough aseptic precautions. The leg was then bandaged and a prophylactic dose of ampicillin (Pen & Strep®-Norbrook Pharmaceutical Co., Ltd.) 20 mg/kg IM for 3 successive days was given. All horses were kept in stable confinement for 2 weeks and thereafter subjected to a rehabilitation program by daily mild hand walking for 15–30 minutes until the end of the follow-up period (8 weeks). All patients were subjected to clinical and ultrasonographic evaluation before therapy and at 2, 4, and 8 weeks post injection. Clinically, lameness grading was modified from (*Stashak* 2002) and classified using a qualitative score from 0 to 3 (0 = no lameness, 1 = mild degree of increase in lameness, 2 = moderate lameness, and 3 = severe lameness) (Table 1). Furthermore, the overall grades of post therapeutic tendon recovery were scored as good (+++), fair (++), and poor (+), according to (*Gillis* 1997).

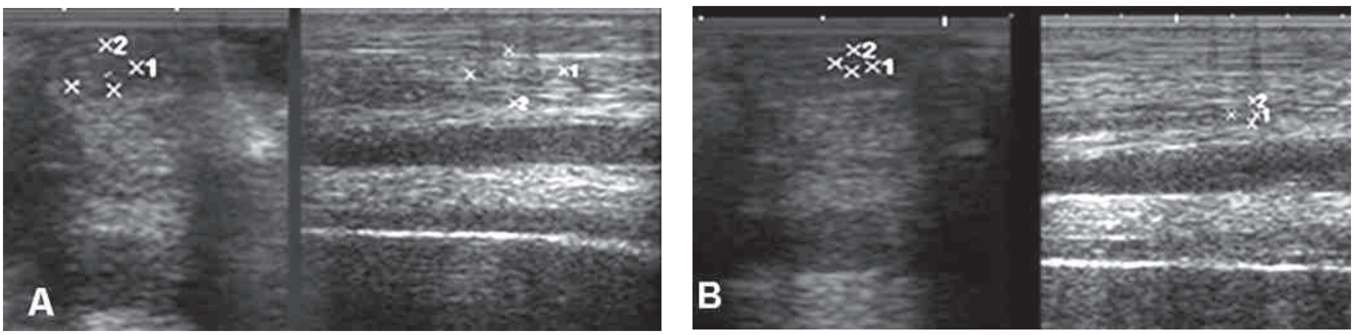
- Good (+++)= the horse returned normal with complete competitive condition.
- Moderate (++)= the horse returned normal with poor competitive condition.
- Poor (+)= the horse returned normal without competitive ability.

Results

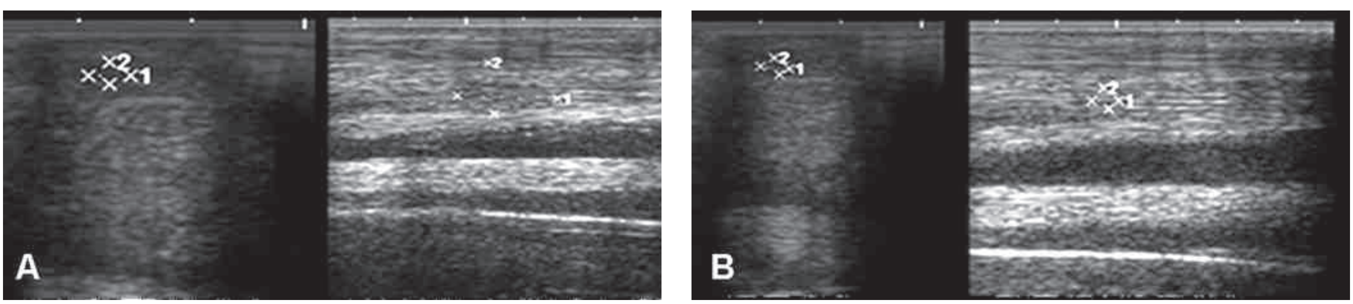
Among the enrolled 15 athletic horses, there were 7 males and 8 females of mixed breeds (age range, 4–12 years and weight range, 350–450 kg). Follow-up clinical examination revealed mild local swelling, pain, heat, and mild to moderate lameness for a few days after MSCs therapy; however, these signs gradually disappeared with relative improvement in the tendon lesion parameters by the 2nd week. Follow-up ultrasonographic examinations revealed marked improvement in their parameters by the 4th week of MSCs therapy (Fig. 2, Table 2). By week-8, the original tendon lesion was no longer detected in 7 cases out of 15 cases, represented (46.6 %) with associated lack of local clinical signs. The rehabilitation plan was started after 2 days from the MSCs therapy. Seven



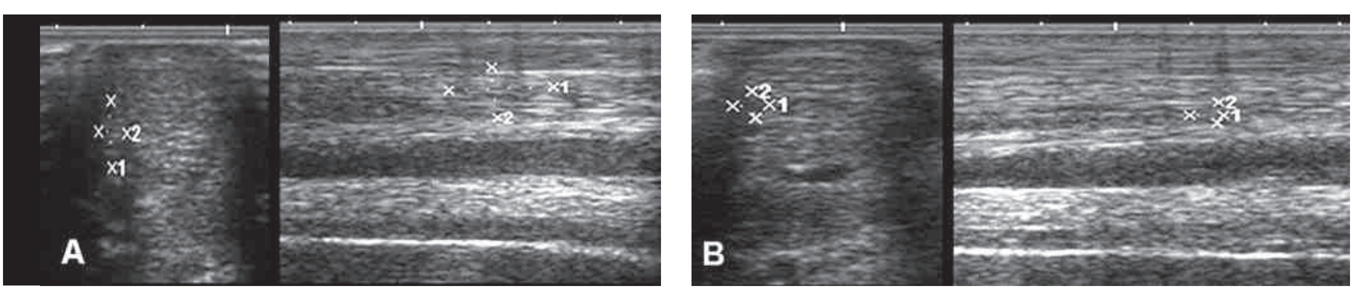
Case No. :3 8-year old gelding Hunter with SDFT tendonitis. A) Before therapy B) After 3 weeks from MSCs therapy



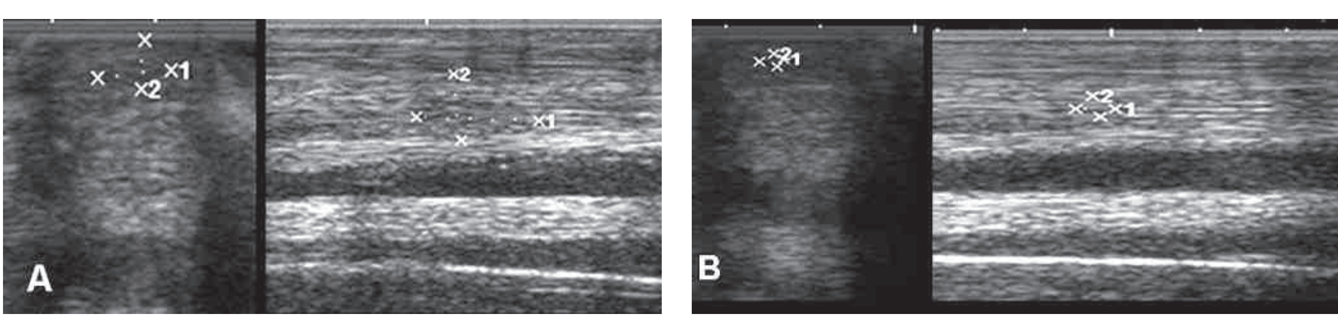
Case No. 6 8-year old gelding Holstein with SDFT tendonitis. A) Before therapy B) After 3 weeks from MSCs therapy



Case No. 7 11-year old mare Holstein with SDFT tendonitis. A) Before therapy B) After 3 weeks from MSCs therapy



Case No. 13 11-year old gelding Oriental with SDFT tendonitis. A) Before therapy B) After 3 weeks from MSCs therapy



Case No. 14 6-year old mare Hunter with SDFT tendonitis. A) Before therapy B) After 3 weeks from MSCs therapy

Fig. 2 Sonograms of some clinical cases before and after 3 weeks from MSCs treatment cases of the presented treated cases showed a marked success rate and started to share in their normal sport activities after 8 weeks. Three other cases (20%) were gradually showing recovery by time after MSCs implantation without any marked adverse reaction, however, they were not rolled in any risky sport activity as extra-habilitation time was needed. Meanwhile, five of the presented cases, exhibited moderate slowly recovery and therefore, they were not adapted for rehabilitation list recurrence of the lesion. A likely explanation of such delay in recovery of these 5 cases, is the injury of a relatively large area of the tendon structure. Overall, the majority of the MSCs treated clinical cases (66.7%) showed acceptable recovery after a somewhat short rehabilitation time of 4 weeks and approximately half of these horses (7 cases) have returned to their usual athletic activities by week-8 (Figs. 3&4).

Discussion

In the present clinical study, the technique adopted by (*Kasashima* et al. 2011) of sternal bone marrow aspiration from the 5th sternebra was found practical and safe for collecting the optimum amount of bone marrow. Also, pre-skin short incision breach was necessary to avoid the agitation of the horse by the sudden puncture of Jamshidi needle through the skin which might deviate the direction of the needle to a risky position. The pre-marking of the needle's shaft to 2 cm from the tip length was essential for

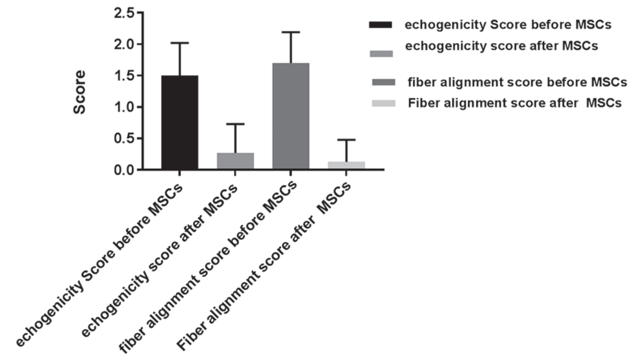


Fig. 3 Mean ± SD of echogenicity and fiber alignment scores of SDFT cases of the clinical model before and after MSCs treatment.

Table 2 Clinical and ultrasonographic evaluation of clinically affected horses before and at the 8th week of autogenous MSCs therapy.

Patient no.	Age (year)	Sex	Lameness score (at initial presentation)	US evaluation before MSCs implantation			Rehabilitation duration (week)	US evaluation after MSCs implantation			Overall therapeutic evaluation score
				Echogenicity score	Fiber alignment score	MIZ %		Echogenicity score	Fiber alignment score	MIZ %	
1	12	M	1	1	2	28	3	0	0	3	+++
2	8	F	1	1	2	29	3	1	1	8	+
3	8	F	1	1	2	25	3	0	0	3	+++
4	11	F	2	2	2	38	4	0	0	7	++
5	4	F	2	2	2	36	4	1	1	13	+
6	8	M	1	1	1	22	3	0	0	6	++
7	11	M	1	1	1	23	2	1	0	5	+
8	6	F	1	2	2	27	2	0	0	4	+++
9	11	F	1	2	2	26	2	0	0	3	+++
10	7	M	2	2	2	39	4	1	0	12	+
11	6	M	1	1	1	19	2	0	0	4	+++
12	9	M	2	2	2	36	4	0	0	12	+
13	11	F	1	1	1	19	2	0	0	4	++
14	6	F	1	1	1	20	2	0	0	4	+++
15	6	M	1	2	2	25	2	0	0	5	+++

- Lameness scores in (Table 1)
- Tendon lesion area (maximum injured zone, MIZ%) = lesion area/tendon area X 100.
- Echogenicity score: 0 = Normal echogenicity (< 25% loss of echogenicity); 1 = 25–50% loss of echogenicity; 2 ≥ 50% loss of echogenicity; 3 = Mostly to completely anechoic.
- Fibers alignment score: 0 = Aligned/parallel pattern in > 75% of the fibers; 1 = Parallel pattern in 51–75% of the fibers; 2 = Parallel pattern in 25–50% of the fibers; 3 = Parallel pattern in < 25% of the fibers.
- Rehabilitation duration: controlled exercise (walking and trotting) for 15–30 minutes daily.
- (+++): the horse returned to normal with complete competitive condition. (++): the horse returned to normal with poor competitive condition.(+): the horse returned to normal without competitive ability.

limiting further sternal penetration of the needle. Aspiration of 10 ml was found sufficient for culturing and therapy. Other alternative site to aspirate BM is the tuber coxae that was used by (*Russell* et al. 1994). Auto-implantation of 1 ml (10×10^6) was within the range used by (*Fortier* and *Smith* 2007, *Smith* and *Webbon* 2005). The overall findings indicate a great benefit to the use of MSCs therapy for the treatment of acute tendonitis of the SDFTs with promising results whereas the majority of the MSCs treated clinical cases (46.7 %) showed recovery after a somewhat shortened rehabilitation time of 4 weeks and higher number of horses returning to the usual athletic activities. Meanwhile, (33.3 %) of the presented cases, treated with MSCs exhibited moderate recovery and required more time for rehabilitation. That means 80 % of the presented cases showed the potential of MSCs therapy for successful treatment of acute tendonitis. Also, 9 out of 11 animals treated with MSCs showed full recoveries as shown by ultrasound images of the tendons taken after a period of 3–6 months, when they returned to racing. Similarly, (*Smith* et al. 2003) treated an 11-year-old polo pony that had injury of the SDFT 5 weeks previously using autologous MSCs implantation into the tendon lesion with subsequent marked improvement after 6 weeks from implantation. Ultrasonography has proven its efficacy as a predictory tool for prognosis, as determined by MIZ % and echgenicity and fiber alignment scores as reported by (*Genovese* et al. 1990, *Reef* 2001, *Barreira* et al. 2008). These cells have the potential to produce actual tendon matrix rather than poorly functional star tissue, as occurs with conventionally treated SDF tendopathy. MSCs therapy for tendon and ligament injuries provides higher diposition of type I collagen with subsequent collagen fiber pattern organization which are prerequisite for returning to athletic activities (*Dahlgren* 2009, *Smith* 2010. Furthermore, MSCs implantation into the tendon lesion promoted anti-inflammatory effect and liberation of growth factors which contribute to tendon repair (*Caplan* 2005). No adverse effects demonstrated following MSCs tendon implantation. This is consistent with the results of (*Godwin* et al. 2011). According to (*Smith* et al. 2003), the optimum time for the implantation of MSCs is unclear, therefore further studies should be conducted to clear this point, which is currently standardized between 7 and 45 days after the onset of the lesion (*Carvalho* et al. 2013). Overall, the MSCs therapy should optimally be done within a month of the injury before scare tissue becomes established. Furthermore, rehabilitation using a controlled exercise program is necessary to give the tendon the chance to heal to full strength; otherwise recurrence of tendon lesion can develop. Nevertheless, as a result of the clinical nature of the present study, the use of a placebo group was not possible, and a future experimental study is therefore warranted to overcome this particular study limitation.

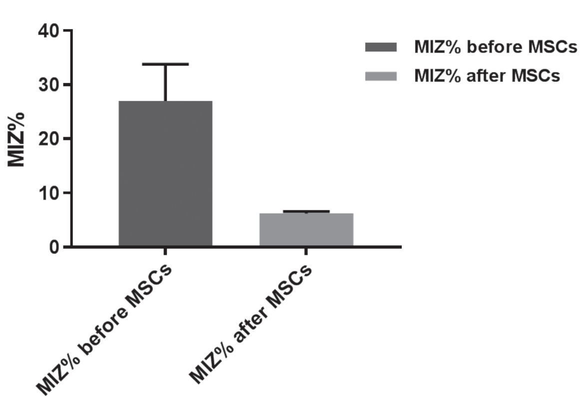


Fig. 4 Mean ± SD of MIZ % of SDFT cases of the clinical model before and after MSCs treatment

Conclusions

Based on the clinical and ultrasonographic outcomes identified in the present study, the use of MSCs therapy has proven its efficiency for tendon repair and contribute to reduce the recovery period and subsequent rapid return to athletic activity.

Conflict of interest

The authors declare no conflicts of interest and no competing financial interests related to this article.

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