



## Effect of Hyaluronic Acid Gel and Bone Marrow Topical Applications on Healing of Tenotomized Achilles Tendon in Dogs: Clinical, Ultrasonography, Histopathology and Immunohistochemistry Evaluations



F. M. Mohammed<sup>1</sup>, L. M. Alkattan<sup>1\*</sup>, O. M. Al Iraqi<sup>2</sup> and Nazhad H. Qader<sup>3</sup>

<sup>1</sup>Department of Surgery and Theriogenology, College of Vet. Med. University of Mosul, Iraq.

<sup>2</sup>Department of Internal Medicine, College of Vet. Med. University of Mosul, Iraq.

<sup>3</sup>University of Salahdein, Erbil, Iraq.

**T**HIS STUDY evaluated the impact of hyaluronic acid gel (HA) and bone marrow (BM) on healing of tenotomized Achilles tendon in dogs. Under general anesthesia, eighteen adult local dogs were enrolled randomly into three equal groups (6 dogs each). All animals were subjected the same condition of accommodation. Right hind limb at Achilles tendon was prepared aseptically. A linear incision (3-5 cm in length) was carried out over the skin directly at the site of proximal part of Achilles tendon, then the tendon resected transversely. The ends of resected tendon were sewed with 3/0 nylon suture material. The dogs were divided into 3 equal groups according to treatment; first group as control, second group (treated with HA) and third group (treated with BM). Clinical, ultrasonographical and histopathological assessments were done during (15,30, 60 days). Clinically, there was no signs of inflammatory reaction or severe lameness along the period of treatment. Ultrasonographic investigations results in HA and BM groups at 60 days indicated normal echotexture pattern of the tendon with superior changes in BM group in which the tendon returned to the normal feature. Histopathological results at 60 days in BM group showed highly vasculature and mature collagen fibers. Immunohistochemical results of IL-6 at 15 days in BM group indicated intense positive expression in this group superior than the other groups. In conclusion the impact of BM on healing progressing of tenotomized Achilles tendon in dogs is superior to the impact of HA regarding the clinical, ultrasonography, histopathology and immunohistochemistry findings.

**Keywords:** Hyaluronic Acid, Bone Marrow, Tendon defect, Ultrasonography, Immunohistochemistry Dogs.

### Introduction

The inadequate blood flow and a significant risk of adhesion, which restricts the tendon's active and passive mobility, the healing progress very slowly [1].

Dog Achilles tendon diseases typically have a traumatic origin. The extent of the damage may differ greatly depending on the impact, resulting in minor or major injuries, stretching, or a whole rupture. Furthermore, systemic

illnesses may cause a tendon's structure to weaken or burst [2]. Although tendon tissue is ineffective at successfully repairing itself, tendon tissue ruptures frequently result in a three separate phases process of healing [3]. Research on bioactive materials and modified biological or synthetic implants and scaffold is presently underway to improve healing process of many tendon affections as fascia lata [4], pericardium membrane [5], ovine and porcine small intestine submucosa [6], venous graft and plasma rich

\*Corresponding author: L. M. Alkattan, E-mail: laythalkattan@yahoo.com, Tel.: +964 7740850658

(Received 08/01/2024, accepted 18/03/2024)

DOI: 10.21608/EJVS.2024.261513.1773

©2024 National Information and Documentation Center (NIDOC)

platelets [7,8] polypropylene mesh and synovial fluid [9], bone marrow [10] hyaluronic acid [11]. All previous studies mentioned materials as bioactive materials. Hyaluronic acid (HA) is an essential part of articular cartilage is attached to aggrecan monomers that give strength power to cartilage and contribute in absorbing water [11]. The therapeutic applications of HA including therapy of tendinopathies its viscoelastic and anti-inflammatory capabilities [12], so it enhance function and reduce pain in a therapeutic setting [13]. Also, another studies reinforce its application of HA, its plays crucial role as a scaffold in bone grafting processes and healing [14], Improved tendon healing [15] and colonic anastomosis [16]. The bone marrow is play important role in healing of defective tissues and organs such as trachea [17], esophageal anastomotic [18] and enhancement healing of bone tissue [19]. In veterinary medicine, ultrasonographic examination of Achilles tendon injury is the preferred diagnostic method for incomplete tears since it allows for direct view of tendon architecture [8]. This study designed to assess the impact both the hyaluronic acid gel and bone marrow to activate and enhance the healing process of experimentally tearing of Achilles tendon in dogs.

### **Material and Methods**

Adult 18 dogs of stay dogs were used in suggested experiment their weight and age were (22±0.4) kg, (2±0.8) years respectively. The experiment done according to Animal Care and Committee procedures and approved protocols at the University of Mosul, College of Veterinary Medicine No: UM.Vet. VET.2023.052. Under protocol general anesthesia using mixture of Ketamine hydrochloride 10% (Dutchfarm, Holland) and Xylazine hydrochloride 2% (Interchemi, Holland) intravenous injection at a dose (3 mg/ kg B.W., 15 mg/ kg Bwt.), respectively premeditated with Atropine sulphate (Vapco) subcutaneously at a dose of (0.04 mg/ kg B.wt.) [20]. All experimental animals underwent same condition of accommodation and feeding in the animals house of Veterinary College University of Mosul. This experiment divided randomly into three equal group according to its treatment received. Right hind limb was prepared aseptically, 3-5 cm over the skin directly at the site of Achilles tendon proximal to the hock joint, then the tendon exteriorized and resected transversely, then the ends of resected tendon was sewed with 3/0 nylon suture material with

far-near-near-far suture technique, experiment enrolled into three groups, first as control, second treated with 1ml of 1% hyaluronic acid gel which spread directly at the operative site (Fig. 1) finally, the subcutaneous tissue closed by simple continuous suture technique and skin sutured by horizontal mattress suture, the third, treated with bone marrow, the bone marrow directly withdrawn from proximal part of the femur bone by using special needle is called Jamshidi needle then the fresh bone marrow prepared 1 to two ml collected and prepared according to [21] the collected BM spreads directly at the site of reconstructed tendon (Fig. 2). The operative leg fixed by plaster of Paris with small window for continues irrigated and the wound dressed, using wound spray, antipyretic drug 1 ml /10kg Bwt. antibiotic penicillin –streptomycin at dose rate 1 ml /10kg intramuscularly for 5 consecutive days. The plaster of Paris was removed 30 days post-surgery. Clinical, ultrasonographical and histopathological assessments were done at 15, 30, 60 days post-surgery.

Clinical observations included daily inspection for local heat, swelling, lameness and inflammatory signs or bacterial infection at the site of operation. Lameness investigated according to the lameness scoring (1 to 4 scale) system [22].

Grade – Score system of gait categories (Spinella *et al.*, 2021)

Grade -Score	Gait categories
0	Normal gait
Mild -1	Weight-bearing lameness
Moderate - 2	Weight-bearing lameness intermittent non-weight bearing
Severe - 3	Non-weight-bearing lameness with brief intermittent weight-bearing
More severity 4	Non weight-bearing lameness at all time

**Statistical analysis**: t test analysis was used to detect the significant difference (Analysis of Variation-ANOVA one way) at  $P \leq 0.01$

Ultrasound assessment in both treated and controlled were scanned longitudinally on the Achilles tendon site using (7 MHz by linear probe). Using Ultrasound scanner, Zhou Kaixin Electronic Instrument (China). Tissue specimens were collected and the sections were stained with Harri's hematoxylin and alcoholic eosin [23], and the stained slides were examined at 100X.

Immunohistochemistry was done by using the avidin-biotin immunoperoxidase technique. The collected specimens were deparaffinised, rehydrated, deactivated then submitted to IHC protocol. The slides were incubated with primary antibodies for *IL-6* (Cat# E-AB-30095, Elabscience, China) [24].

## Results

The outcome of clinical investigations in this study indicated, normal appetite, there were no any inflammatory reaction or wound dehiscence or severe complications during the treatment period. The signs of severe lameness were evidence in control group which was subsided during few days of treatment and animals retained to the normal activity, whereas there were no signs of lameness in other treated group (Table 1).

Macroscopically the results represented by presence of partial healing with mild degree of adhesion between neighboring tissue and tendon just at defective site at 15 days, then developed to mild to severe adhesion which become a thick and edematous at 30 and 60 days P-S in control group.

At 30 days P-S in treatment group with HA also there were mild degree of adhesion between the Achilles tendon and neighboring tissue (Fig. 3: A) Whereas at same period in group BM the results indicated less degree of adhesion (Fig. 3: B)

At 60 days in BM group postoperatively the outcome indicated no signs of adhesion and presence of a thread with the healing process done completely (Fig. 4).

The results of ultrasonographic investigations at 15 days post treatment in control group indicated loss of arrangement of the fiber pattern with hypoechogenic spots in the center represent fluid accumulation (Fig.5: A) whereas in HA group the outcome indicated presence irregular heterogeneous pattern with hypoechogenic areas in the center represent fluid of the healing process (Fig.5: B), In group BM indicated presence of enlarged and irregular, heterogeneous in echogenicity with hypoechoic foci of Achilles tendon (Fig 5: C).

Thirty days P-S in control group the outcome indicated heterogeneous fiber pattern with net-shape of hypoechogenicity represent fluid (Fig.6:

A). In group HA there was regular, homogenous pattern of the tendon (Fig.6,B), whereas in BM group there were presence homogenous pattern with an area of hyperechogenic (Fig.6,C). At 60 days post-surgery in control group the results reveal normal homogenous fiber pattern (white arrow) with a line of hyperechogenicity represent healing process (Fig.7, A). while in HA group there was regular homogenous pattern (Fig.7,B). Finally In the BM return to normal echo texture pattern of the tendon (Fig 7: C).

Histopathologically at 15 days postoperatively in control and HA group exhibited presence granulation tissue with high inflammatory cells and regeneration of the collagen fibers surrounding the operative area (Fig.8-A, B), the collagen fibers well developed than control whereas in treatment group with BM the result represented by presence mild granulation tissue with mild inflammatory cells infiltration and very well-developed collagen fibers (Fig.8-C). At 30 days postoperatively in the control group showed mild granulation tissue and well-developed collagen fibers (Fig 9-A). At the same period in the treated group with HA, the site of operation was completely occluded by regenerated immature collagen fibers with new blood vessels in BM group (Fig 9: B, C). In the control group, the site of operation was surrounded by mild granulation tissue and regenerated immature collagen fibers (Fig. 10-A) whereas the treated group with HA showed the site of cut operation completely occluded by regenerated immature collagen fibers with few inflammatory cells (Fig. 10- B) finally in the treated group with BM the site of operation completely occluded by regenerated immature and mature collagen fibers with vasculature (new blood vessels) (Fig.10-C).

Immunohistochemistry assessment results of *IL-6* for dog tendon of the BM represented intense positive expression during first few days of treatment ( Fig.11: A), whereas the gene expression of treated animals in HA group there was moderate positive reaction which exhibited as golden-brown granules in cells cytoplasm (Fig.11: B) .In control group, 15 days P-S there was mild positive expression of *IL-6* for dog tendon indicated as golden-brown granules in cytoplasm of cells (Fig 11: C).

**TABLE 1. Grade – Score system of gait categories.**

HA group	BM group	Control group *	Days / Grade- Score
2.00 ± 0.8 <sup>b</sup>	1.33 ± 0.27 <sup>c</sup>	2.83 ± 0.57 <sup>a</sup>	<b>1 day</b>
0.86±0.81 <sup>c</sup>	0.00±0.00 <sup>d</sup>	1.29±1.24 <sup>c</sup>	<b>7 days</b>
0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>d</sup>	<b>15 days</b>

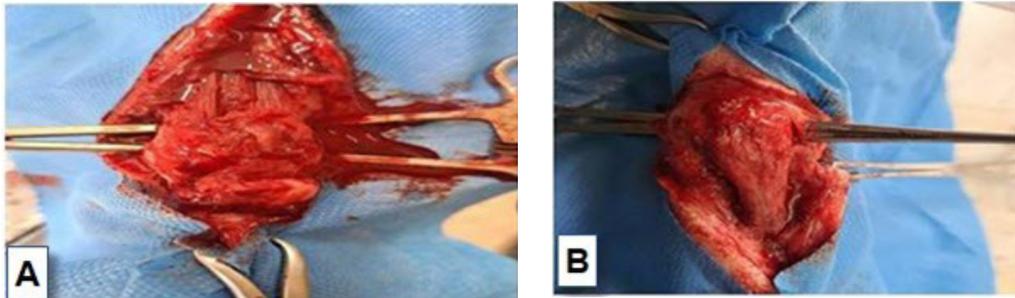
\* Different letters mean a significant at  $p \leq 0.01$



**Fig. 1. Image shown repairing the induced completely cutting Achilles tendon, the site of operation treated with 1m of 1%hyaluronic acid gel in the second group.**



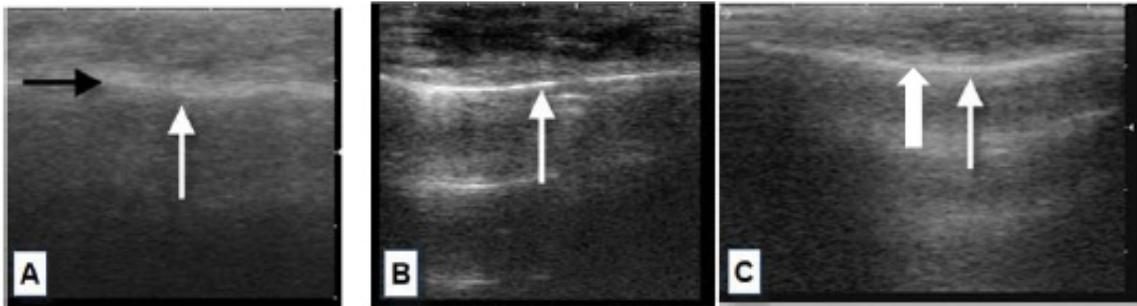
**Fig. 2. Image shown repairing the induced completely cutting Achilles tendon, the site of operation treated with 1m of bone marrow in the third group.**



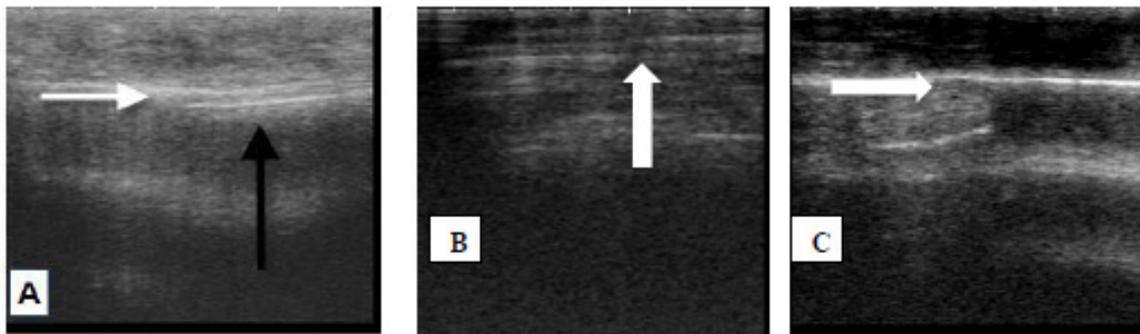
**Fig. 3. Macroscopical image of treated group with HA at 30 days postoperatively shows presence of a mild degree of adhesion between the Achilles tendon and neighboring tissue (Fig. 4,A) Whereas at same period in group BM the results indicated less degree of adhesion (Fig.4, B).**



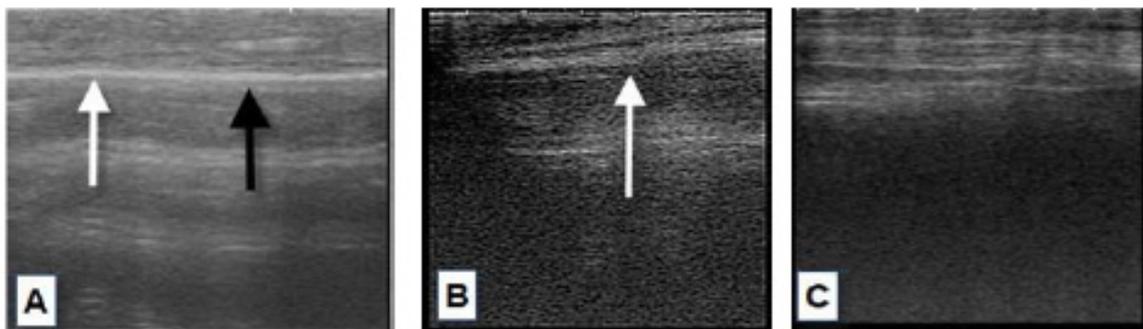
**Fig. 4. Macroscopical image of treated group with BM at 60 days postoperatively shows with complete healing of a cutting tendon ends.**



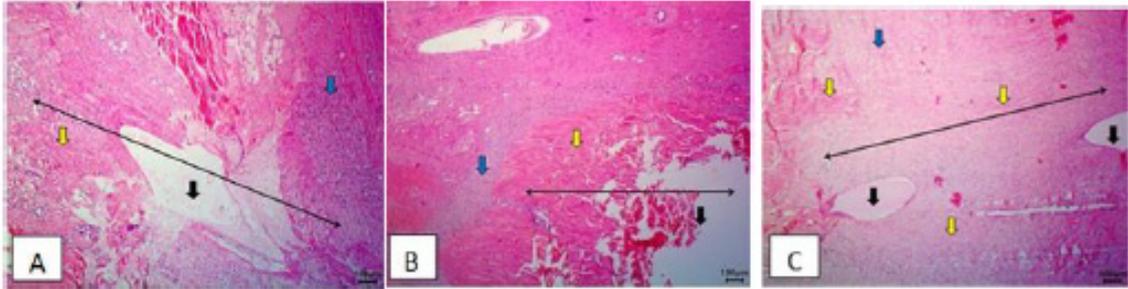
**Fig. 5.** A). Ultrasonographic image directed longitudinally at the lateral aspect of the Achilles tendon in the control group at 15 days postoperatively represents the presence of loss of arrangement of the fiber pattern (black arrow) with hypoechogenic spots (white arrow). B). Ultrasonographic image directed longitudinally at the lateral aspect of the Achilles tendon in the HA group at 15 days postoperatively represented the presence of an irregular heterogeneous pattern with hypoechogenic areas (white arrow). C). Ultrasonographic image directed longitudinally at the lateral aspect of Achilles tendon in BM group at 15 days postoperatively represent presence of enlarged and irregular, heterogeneous in echogenicity with hypoechoic foci of Achilles tendon (white arrow).



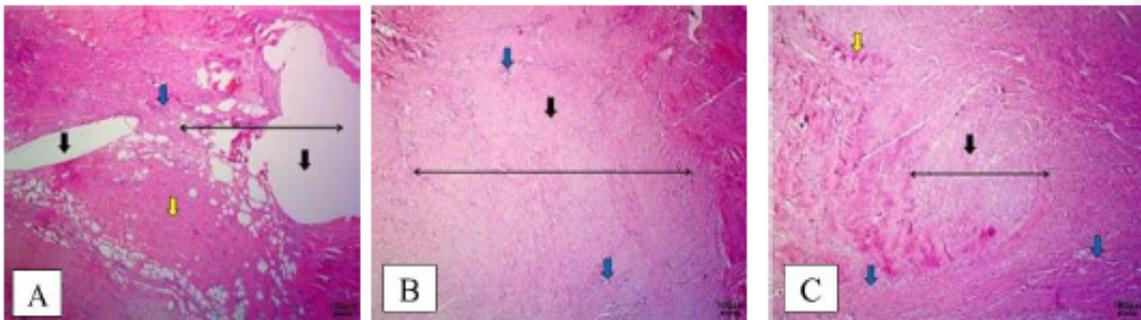
**Fig. 6.** (A) Ultrasonographic image directed longitudinally of the lateral aspect of Achilles tendon in the control group at 30 days postoperatively representing the presence of heterogenous fiber pattern (black arrow) with net-shape of hypoechogenicity (white arrow) representing fluid. B). Ultrasonographic image directed longitudinally of the lateral aspect of the Achilles tendon in the HA group at 30 days postoperatively indicated the presence of a regular, homogenous pattern of the tendon (white arrow). C). Ultrasonographic image directed longitudinally of the lateral aspect of Achilles tendon in the BM group at 30 days PS represents a homogenous pattern with an area of hyperechogenic.



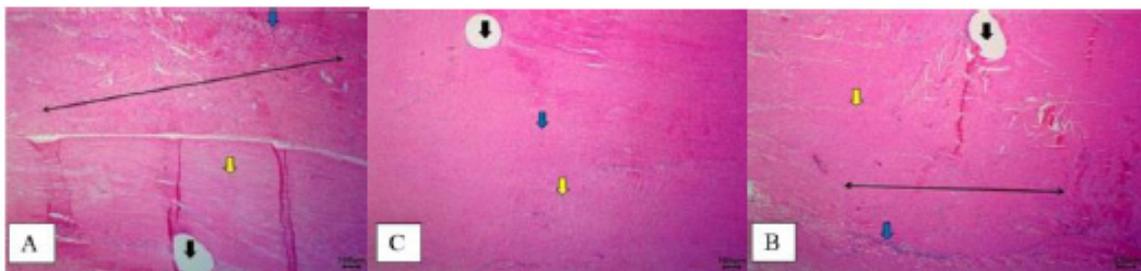
**Fig.7.** A) Ultrasonographic image directed longitudinally of the lateral aspect of Achilles tendon in the control group at 60 days P-S represents a normal homogenous fiber pattern (white arrow) with a line of hyperechogenicity (black arrow) representing a healing process. B) Ultrasonographic image directed longitudinally of the lateral aspect of Achilles tendon in the (HA group at 60 days P-S represents a regular homogenous pattern. C). Ultrasonographic image directed longitudinally of the lateral aspect of Achilles tendon in the BM group at 60 days P-S represent return to normal echotexture pattern of the tendon.



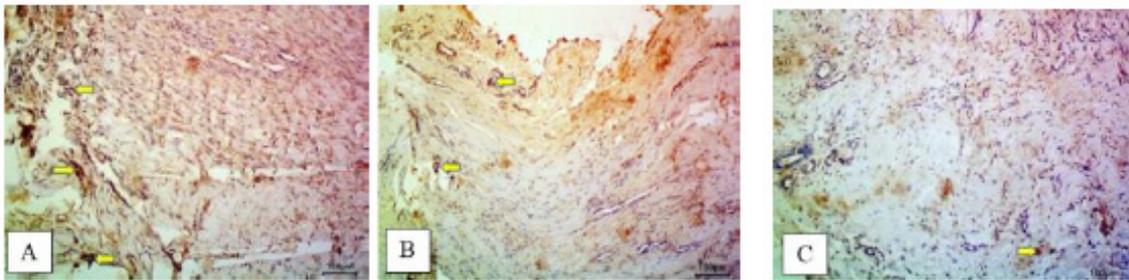
**Fig. 8.** A). Photomicrograph for tendon dog of the control G (15 days) showing the site of operation (↔), peritendinous space (black arrow), surrounding by granulation tissue with high inflammatory cells (blue arrow) and regeneration of the collagen (yellow arrow). H&E stain, 40X. B). Photomicrograph for the tendon dog of the HA group (15 days) showing the site of cut operation (↔), peritendinous space (black arrow), surrounding by granulation tissue with inflammatory cells (blue arrow) and well-developed collagen (yellow arrow). H&E stain, 40X. C). Photomicrograph for tendon dog of the BM group (15 days) showing the site of cut operation (↔), suturing space (black arrow), occluded by mild granulation tissue with mild inflammatory cells infiltration (blue arrow) and very well-developed collagen (yellow arrow). H&E stain, 40X.



**Fig. 9.** (A). Photomicrograph for the dog tendon of the control group (30 days) showing the site of cut operation (↔), peritendinous and suturing space (black arrow), surrounding by mild granulation tissue (blue arrow) and well-developed collagen fibers (yellow arrow). H&E stain, 40X. B). Photomicrograph for the tendon dog of the HA group (30 days) showing the site of cut operation (↔) completely occluded by regenerated immature collagen fibers (black arrow) with new blood vessels (blue arrow). H&E stain, C). Photomicrograph for the tendon dog of the BM treated group (30 days) showing the site of cut operation (↔) completely occluded by regenerated immature collagen fibers (black arrow) with highly vasculature (new blood vessels) (blue arrow) and mature collagen (yellow arrow). H&E stain, 40X.



**Fig. 10.** (A) Photomicrograph for the dog tendon of the control group (60 days) showing the site of cut operation (↔), suturing space (black arrow), surrounding by mild granulation tissue (blue arrow), and regenerated immature collagen fibers (yellow arrow). H&E stain, 40X. B) Photomicrograph for the dog tendon of the Hyaluronic acid HA treated group (60 days) showing the site of cut operation (↔) completely occluded by regenerated immature collagen fibers (black arrow) with few inflammatory cells (blue arrow). H&E stain, 40X. C). Photomicrograph for the dog tendon of the Bone marrow BM treated group (60 days) showing the site of cut operation (↔), suturing space (black arrow) completely occluded by regenerated immature and mature collagen fibers (blue arrow) with vasculature (new blood vessels) (yellow arrow). H&E stain, 40X



**Fig. 11. A).** Immunohistochemical expression of IL-6 for dog tendon of the BM group (15 days) revealing intense positive expression (arrows) (score 3+); 100X. **B).** Immunohistochemical expression of IL-6 for dog tendon of the Hyaluronic acid HA treated group (15 days) revealing moderate positive expression (arrows) (score 2+); 100X. **(C)** Immunohistochemical expression of IL-6 for dog tendon of the control group (15 days) revealing mild positive expression (arrow) (score 1+); 100X.

## Discussion

The surgery for Achilles tendon ruptures and tendinopathies represents a crucial challenge, so adhesion between the tendon and its sheath post-surgery represents the real challenge and problem restricting tendon gliding and proper treatment. Many synthetic and natural or bioactive materials are used to overcome tendon problems [25]. In the control group, the non-weight bearing lameness on the treated leg suggested the inflammatory reaction and local pain at the site of operation, so weight bearing was scored as poor, whereas in the group (BM, HA), reduced inflammatory reaction and pain generation due to the secretion of growth factor lead to rebuilding the defective tendon this results also considers with [26].

This study's clinical ultrasonographic, histopathological, and immunohistochemical investigation demonstrated the beneficial value of applying different biomaterials (BM, HA gel) in reinforcing reconstituted Achilles tendon damage. The improvement in the healing process after applying BM is represented by early retention of normal activity. This might be due to the containment of bone marrow cells [27].

In the current study, there were signs of lameness, redness, and swelling in the control group may be due to the manipulation of the tendon, which lead to local pain and acute inflammation this is represented in another study [28] Also, HA and BM play role in decreasing the pain and inflammation and improve tendon healing this agree with [29]. The BM has immunity characteristics, self-renew, and anti-microbial properties, as well as differentiation and

expansion, and this also agrees with [30], who said that the local application of high molecular weight HA in high concentrations between an injured tendon might be accelerated healing process of tendon and reduce the degree of adhesion.

In the control group, evidence of the presence of a severe degree of adhesion between the Achilles tendon and surrounding tissue, while results indicated mild adhesion in the HA group and absence in the BM group this outcome is considered with a previous study [31] stated that the HA contributed in reducing of adhesion degree and activated gliding properties of tendon and as well as to improve the tendon's architectural organization [32] also using BM contributed in reducing the incidence of adhesion and inflammatory response, the BM have cytokines, and growth factors, as well as stem cells, played important role in the fast inflammatory response and accelerate the healing process of many living tissues as a bone this outcome agree with [33]. Bone marrow graft is an osteogenic cellular implant that has osteoinductive properties comprising pluripotent stem cells, growth factors, and cytokines, which all stimulate bone [33] other workers discuss that the HA, being an effective soft tissue lubricant, might decrease the new extracellular matrix formation due to the inhibition of mononuclear phagocytes and lymphocytes [34].

Ultrasonography of the Achilles tendon of a dog is considered a real challenge for the canine high-resolution with a special linear transducer and attention to transducer position. A curvilinear transducer is not perfect for the investigation of straight tendons because the variable angle of insonation makes the tendon appear falsely

hypoechoic toward the edges of longitudinal images [35]. Sonographic findings consistent with tendon pathology may include changes in fiber pattern, echogenicity, and cross-sectional area [36]. At 15 days postoperatively, the control group exhibited loss of arrangement of the fiber pattern with hypoechoic spots in the treated group with HA revealed an irregular heterogeneous pattern with hypoechoic areas, and the treated group of BM showed the presence of enlarged and irregular, heterogeneous in echogenicity with hypoechoic foci of Achilles tendon as compared with the control group. The hypoechoicity and heterogeneous pattern might explain either due to intra-tendinous edema, which is an indication sign of hemorrhage and inflammatory reaction [37]. The presence of heterogeneous fiber pattern was also prominent, and this could be considered as a clear sign of an intra-tendinous defect, which might also indicate the presence of edema between the tendon fibers at the day 15th P-S, the acceleration in healing progress of tendon was noted sonographically in all groups, especially in BM treated group. The progress in healing could all be attributed to the increased formation and organization of the mature collagen, the echogenicity have also been used to evaluate patient responses to treatment this outcome also consider with previous workers [38]. At 30 days P-S there was a difference between groups in relation to the persisted hypoechoicity, which was associated with decreased tendon inflammation in all groups except in the control group which was showed the presence of a heterogeneous fiber pattern with net-shape of hypoechoicity, and this attributed to the presence of edema, hemorrhage, inflammatory reaction and an intra-tendinous tear at the site of operation while In the treated group with HA, BM there was regular, homogenous pattern of the tendon and with an area of hyperechogenic in BM group. Regions of hyperechogenicity that were observed in the HA and BM groups revealed evidence of connective tissue formation, scar tissue, chronicity, or intra-tendinous calcification, which may be recovered gradually [39]. However, as the presence of hyperechoic foci is also indicative of intratendinous calcification, a Homogenous fiber pattern was observed only in the HA and BM-treated group, which might be indicative of mature connective tissue formation [36]. At 60 days postoperatively, the improvement in tendon healing was particularly observed in the BM group, while in the control group, the ultrasonography indicated a normal homogenous

fiber pattern with a line of hyperechogenicity representing the healing process. The HA group showed a regular homogenous pattern. Finally the BM returns to the normal echo texture pattern of the tendon, and this is attributed to MSCs cells may differentiate into tenocytes, directly contributing to tendon regeneration [40]. The MSCs contributed directly to growth factors and cytokines releasing that recruit circulating stem cells to the site of defect, increase collagen density, stimulate new blood vessel formation, and inhibit apoptosis and fibrosis, facilitating restoration of normal tendon architecture and its strength [41].

The results of histopathological examinations at 15 days postoperatively represented the presence of mild granulation tissue, mild inflammatory cell infiltration, and very well-developed collagen fibers in the BM group compared with the HA and control group, which agrees with [42]. At 60 days postoperatively, the outcome represented by occlusion of regenerated immature and mature collagen fibers with the vasculature (new blood vessels) in the BM group as compared with HA and control group, and this agrees with [19] who mentioned that the MSCs have the ability to differentiate into various types of cells involving tenocytes, chondrocytes, adipocytes, osteoblasts, and bone marrow stromal cells. Also can be distinguished into chondrocytes and fibroblasts and have the ability to form connective tissues [42,43]

The interleukin (IL-6) activates collagen fiber deposition, and it plays a crucial role in the first few days of healing after declining level the days then, the IL-12 concentration in the wound site increases (post-inflammatory cytokines) in this case, rapid granulation tissue formation occurs, the maturation deposition of collagen developed these events lead to shortening time wound healing this events appear clearly in group BM. Also (IL-6) activates the proliferation of tendon-derived stem cells, positively impacting tendon healing. [35]

### **Conclusion**

In this manuscript, outcome results suggested that the repaired tendon reinforced with HA and BM has a regenerative impact in reconstructing tendon defects, but the superiority is for tendons reinforced with bone marrow.

### **Acknowledgment**

This study was supported by the Surgery and Theriogenology Department. The College of Vet. Med., Mosul University, Iraq.

Conflicted interest: No Conflicted interest.

Funding statement: self-funding

*Ethical approval:* The experiment done according to Animal Care and Committee procedures and approved protocols at the University of Mosul, College of Veterinary Medicine No: UM.Vet.VET.2023.052.

*Author contribution:* The workers designed the research idea and prepared the practical part of the research, while conducting statistical analysis, writing, and final review of the research.

## References

- Jahani, S., Moslemi, H.R. and Dehghan, M. The effect of butyric acid with autogenous omental graft on the healing of experimental Achilles tendon injury in rabbits. *Iranian J. of Vet. Res.*, **16**(50),100-104(2015)
- Montgomery, R. and Fitch, R. Muscle and tendon disorders. In: Slatter D (ed): Textbook of Small Animal Surgery. 7<sup>th</sup> ed. Saunders, Philadelphia. 2266–2267. (2003)
- Docheva, D., Muller, S.A., Majewski, M. and Evans, C.H. Biologics for tendon repair. *Adv. Drug Deliv. Rev.*, **84**, 222–239 (2015). doi:10.1016/j.addr.2014.11.015.
- Tang, J.B. Clinical outcomes associated with flexor tendon repair. *Hand Clin.*, **21**(2),19 (2005). doi:10.1016/j.hcl.2004.11.005
- Demirkan, I., Atalan, G., Cihan, M., Sozmen, M. and Kankavi, O. Replacement of ruptured Achilles tendon by fascia lata grafting. *Veteriner. Cerrahi Dergisi.*, **10**, 21–26(2004)
- Dal-Bó, I.S, Ferrigno, S.R, Macedo, A.S, Paes, J.F., Marinho, P.V, Bregadioli, T.H. and Galeazzi, V.S. Tenorrhaphy of the Common Calcaneal Tendon in Dogs and Cats: case report. *Acta. Scie. Vet.*, **44**(11),144(2016). doi:10.22456/1679-9216.84404
- Mohammed, F.M. Using of ovine small intestinal submucosa for repairing experimental defect of Achilles tendon in indigenous dogs. *Al-Anbar J. of Vet. Scie.*, **8**(2), 54-64(2015).
- Allawi, A.H., Alkattan, L.M. and Al Iraqi, O.M. Clinical and ultrasonographic study of using autogenous venous graft and platelet-rich plasma for repairing Achilles tendon rupture in dogs. *Iraqi J. of Vet. Scie.*, **33**(2), 453-460(2019). doi:10.33899/ijvs.2019.163199
- Naeem L.A., Naeem, R.M. and Ibrahim, A.A The efficacy of polypropylene mesh and synovial fluid on tendon healing in dogs :A comparative study. *Eurasia J. Biosci.*, **14**, 7501-7507(2020).
- McDougall, R.A., Canapp, S.O and Canapp, D. A. Ultrasonographic Findings in 41 Dogs Treated with Bone Marrow Aspirate Concentrate and Platelet Rich Plasma for a Supraspinatus Tendinopathy:Retrospective Study. *Front. in Vet. Scie.*, **5**(98) ,1-10(2018). doi.org/10.3389/fvets.2018.00098
- Abate, M., Schiavone, C. and Salini, V. The use of hyaluronic acid after tendon surgery and in tendinopathies. *Biomed. Res. Int.*, **2014**, 783632(2014). doi: 10.1155/2014/783632.
- Kaux, J.F., Samson A. and Crielaard, J.M. Hyaluronic acid and tendon lesions. *Muscl. Lig. Tend. J.*, **5**(4), 264-269(2016). doi: 10.11138/mltj/2015.5.4.264.
- Merolla, G., Bianchi, P. and Porcellini, G. Ultrasound-guided subacromial injections of sodium hyaluronate for the management of rotator cuff tendinopathy: a prospective comparative study with rehabilitation therapy. *Musculoskelet. Surg.*, **97**, Suppl 1, 49-56(2013). doi: 10.1007/s12306-013-0259-y.
- Mohammed, F.M., Alkattan, L.M, Shareef, A.M. and Ismail, H.K. The role of adding hyaluronic acid in the grafting process for the repair of an experimentally induced tibial defect in dogs' model. *Iraqi J. Vet. Scie.*, **36**(3),555-561(2022). doi: 10.33899/ijvs.2021.130889.1891
- Oryan, A., Moshiri, A., Meimandi Parizi, A.H. and Raayat Jahromi, A. Repeated administration of exogenous Sodium- hyaluronate improved tendon healing in an in vivo transection model. *J. Tissue Viability*, **21**, 88-102(2012).doi: 10.1016/j.jtv.2012.06.002.
- Lima, I., Azevedo, I., Rocha, K. and Mederos, A. Effect of hyaluronic acid on the healing of colonic anastomosis in rats treated with corticoid. *J. Surg. Cl. Res.*, **12**, 81-92 (2020) . doi:10.20398/jscr.v11i2.23395.
- Al-Hyani, O.H. Evaluation of Different Implants for Repairing of Tracheal Defect in Dogs. Thesis College of Veterinary Medicine, University of Mosul . Mosul .Iraq.(2019)
- Xiang, X., Yan, Y., Ye Ma., Yang, Y., Chunguang, L., Xilong, L., Zhiyun, X., Hezhong, C. and Hao,

- Z. Stem Cell Therapy for Esophageal Anastomotic Leakage by Autografting Stromal Cells in Fibrin Scaffold. *J. Stem Cells Transl. Med.*, **8**, 548–556(2019).
19. Jäger, M., Jelinek, E.M. and Wess, K.M., Scharfstädt, A., Jacobson, M., Kevy Sh. V. and Krauspee, R. Bone marrow concentrate: a novel strategy for bone defect treatment. *J. Curr. Stem Cell Res. Ther.*, **4**,34-43(2009). doi: 10.2174/157488809787169039.
  20. Alkattan, L.M. and Helal, M.M. Effects of ketamine-xylazine and propofol-halothane anesthetic protocols on blood gases and some anesthetic parameters in dogs, *Vet. World.*, **6**(2)95-99(2013). doi: 10.5455/vetworld.2013.
  21. Tomasian, A. and Jennings, J.W. Bone marrow aspiration and biopsy: techniques and practice implications. *Skeletal Radiol.*, **51**, 81–88(2022). doi.org/10.1007/s00256-021-03882-w.
  22. Spinella, G., Davoli, B., Musella, V. and Dragone, L. Observational Study on Lameness Recovery in 10 Dogs Affected by Iliopsoas Injury and Submitted to a Physiotherapeutic Approach. *Animals (Basel)*. **11**(2), 419(2021). doi: 10.3390/ani11020419. PMID: 33562039; PMCID: PMC7914594.
  23. Suvarna, S.K., Layton, C. and Bancroft, J.D., Bancroft's Theory and Practice of Histological Techniques. 7th ed. Churchill Livingstone Press, USA. P. 12-32. (2013).
  24. Post, G.R., Bell, R.C., Rjoop, A., Lobo, R.H., Yuan, Y. and Post, S.R. Diagnostic utility of interleukin-6 expression by immunohistochemistry in differentiating castelman disease subtypes and reactive lymphadenopathies. *Ann. Clin. Lab. Sci.*, **46**(5), 474-479 (2016) .
  25. Shapiro, E., Grande, D. and Drakos, M. Biologics in Achilles tendon healing and repair: a review. *Current Reviews in Musculoskeletal Medicine*, **8**, 9-17(2015).
  26. Kononovich, N.A, Petrovskaja, N.V. and Krasnov, V.V. Treating dogs with tibial shaft fractures using the transosseous osteosynthesis method according to Ilizarov. *Euro. J. of Companion Anim. Pract.*, **24**(2), 51-58 (2014).
  27. Tepper, O.M., Capla, J.M., Galiano, R.D., Ceradini, D. J., Callaghan, M.J., Kleinman, M.E. and Gurtner, G.C. Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. *Blood*, **105**, 1068–1077(2005). doi: 10.1182/blood-2004-03-1051
  28. Mohammed, F.M. Using of ovine small intestinal submucosa for repairing experimental defect of Achilles tendon in indigenous dogs. *Al-Anbar J. of Vet. Scie.*, **8**(2), 54-64(2015).
  29. Mehrabani, D., Mehrabani, G., Zare, S. and Manafi, A. Adipose-derived stem cells (ADSC) and aesthetic surgery: a mini review. *J. World Plast. Surg.*, **2** , 65–70(2013). PMCID: PMC4238346
  30. Tuncay, I., Ozbek, H., Atik, B., Ozen, S. and Akpinar, F. Effects of hyaluronic acid on postoperative adhesion of tendo calcaneus surgery: an experimental study in rats. *J. Foot Ankle Surg.*, **41**,104-108(2002). doi: 10.1016/s1067-2516(02)80033-3.
  31. Oryan, A., Moshiri, A., Parizi, A.H. and Jahromi, R. Repeated administration of exogenous Sodium-hyaluronate improved tendon healing in an in vivo transection model. *J. Tissue Viability*, **21**(3), 88-102(2012). doi: 10.1016/j.jtv.2012.06.002.
  32. Nishida, J., Araki, S., Akasaka, T., Toba, T., Shimamura, T. and Amadio, P.C. Effect of hyaluronic acid on the excursion resistance of tendon grafts. A biomechanical study in a canine model invitro. *J. Bone Joint Surg. Br.*, **86**, 918-924 (2004) .
  33. Korf-Klingebiel, M., Kempf, T., Sauer, T., Brinkmann, E., Fischer, P., Meyer, G.P., Ganser, A., Drexler, H. and Wollert, K.C. Bone marrow cells are a rich source of growth factors and cytokines: implications for cell therapy trials after myocardial infarction. *European Hear. J.*, **29**, 2851–2858(2008).
  34. Swann, D.A, Bloch, K.J, Swindell, D. and Shore, E. The lubricating activity of human synovial fluids. *Arthritis Rheum.*, **27**, 552-556(1984).
  35. Garcia, T., Hornof, W.J. and Insana, M.F. On the ultrasonic properties of tendon. *Ultrasound Med. Biol.*, **29**,1787–1797 (2003).
  36. Zwingenberger, A., Bengini, L., Lamb, C.R., Mistieri, M.L., Wigger, A. and Canola, J.C. Musculoskeletal system". In: Mattoon JS, Nyland TG, editors. Small animal diagnostic ultrasound. 3rd ed. St. Louis, USA: WB Saunders. p. 517–40. (2015)
  37. McDougall, R.A., Canapp, S.O. and Canapp, D. A. Ultrasonographic Findings in 41 Dogs

- Treated with Bone Marrow Aspirate Concentrate and Platelet Rich Plasma for a Supraspinatus Tendinopathy: A Retrospective Study. *Front. Vet. Sci.*, **5**(98), 1-10 (2018). doi.org/10.3389/fvets.2018.00098.
38. Canapp, S.O., Canapp, D.A., Ibrahim, V., Carr, B.J., Cox, C. and Barrett, J.G. The use of adipose-derived progenitor cells and platelet-rich plasma combination for the treatment of supraspinatus tendinopathy in 55 dogs: a retrospective study. *Front. Vet. Sci.*, **3**, 61(2016). doi: 10.3389/fvets.2016.00061.
39. Arend, C.F., Arend, A.A., da Silva, T.R. and Silfa, T.R. Diagnostic value of tendon thickness and structure in the sonographic diagnosis of supraspinatus tendinopathy: room for a two-step approach. *Eur. J. Radiol.*, **83**(6), 975-979(2014). doi: 10.1016/j.ejrad.2014.02.021.
40. Zaidi, N. and Nixon, A.J. Stem cell therapy in bone repair and regeneration. *Ann. N. Y. Acad. Sci.*, **1117**, 62-72(2007). doi: 10.1196/annals.1402.074.
41. Caplan, A.I., Dennis, J.E., Arnold, I. and Caplan, J.E.D. Mesenchymal stem cells as trophic mediators. *J. Cell Biochem.*, **98** (5), 1076-1084(2006). doi: 10.1002/jcb.20886
42. Aghamir, S.M.R.; Mehrabani, D.; Amini, M.; MoslehShirazi, M.A.; Nematollahi, S.; Shekoohi-Shooli, F.; Mortazavi, S.M. and Razeghian, J.I. The regenerative effect of bone marrow-derived stem cells on cell count and survival in acute radiation syndrome. *World J. Plast. Surg.*, **6**, 111-113(2016).
43. Kadiyala, S., Young, R.G., Thiede, M.A. and Bruder, P. Cultured examine canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. *J. Cell Transplant.*, **6**(2), 125-134(1997).
44. Dittrich, A., Kemper, W.H. and Schaper, F. Systems biology of IL-6, IL-12 family cytokines. *Cytokine Growth Factor Rev.*, **26**(5), 595-602(2015). doi: 10.1016/j.cytogfr.2015.07.002

### تأثير الاستخدام الموضعي لجل حامض الهيالورونيك ونخاع العظم على شفاء وتر العرقوب المقطوع في الكلاب: تقييم سريري و بالموجات فوق الصوتية ونسجي مرضي ومناعي نسجي

فؤاد مؤيد محمد<sup>1</sup> ، ليث محمود القطان<sup>1</sup> ، اسامة موفق العراقي<sup>2</sup> و نهزاد حسين قادر<sup>3</sup>  
<sup>1</sup> فرع الجراحة وعلم تناسل الحيوان كلية الطب البيطري - جامعة الموصل - العراق.  
<sup>2</sup> فرع الطب الباطني والوقائي كلية الطب البيطري - جامعة الموصل - العراق.  
<sup>3</sup> فرع الجراحة - كلية الطب البيطري - جامعة صلاح الدين - العراق.

قيمت هذه الدراسة تأثير جل حمض الهيالورونيك ونخاع العظم على التئام وتر العرقوب المقطوع في الكلاب تحت تأثير التخدير العام استخدم ثمانية عشر كلبا بالغاً من السلالة المحلية وقسم عشوائياً الى ثلاثة مجاميع 6كلاب في كل مجموعه. جميع الحيوانات سكنت في نفس المكان. تم تعقيم القدم اليمنى موضع وتر العرقوب. تم اجراء قطع جراحي خطي بطول 3-5سم وبشكل مستعرض. تم خياطة نهايتي الوتر بخيط نابليون جراحي قياس 0/3. قسمت الحيوانات الى ثلاثة مجاميع حسب المعاملة، المجموعة الاولى اعتبرت لمجموعة سيطرة والمجموعة الثانية عولجت بجل حمض الهيالورونيك والمجموعة الثالثة عولجت بنخاع العظم. تم تقييم الحيوانات سريريا وباستخدام الامواج فوق الصوتية ونسجي مرضي خلال 15,30,60 يوم سريريا لا يوجد علامات التهابيه او عرج شديد خلال فترة التجربة. نتائج فحوصات الامواج فوق الصوتية في مجموعة الهيالورونيك ونخاع العظم في اليوم 60 وجود صدق طبيعي للوتر مع تغيير متميز لمجموعة نخاع العظم وعودة الوتر لشكله الطبيعي اما نتائج الفحص النسجي المرضي فاطهر خلال اليوم 60 انتشار دموي كثيف والياق كولاجين ناضجة. اظهرت فحوصات كيمياء النسيج المناعية فحص الانترلوكين 6 باليوم 15 تعبير ايجابي مكثف وتغلبيت هذه المجموعة على بقية المجاميع وخلاصة ذلك ان تأثير استخدام نخاع العظم على التئام وتر العرقوب بشكل متميز على تأثير حامض الهيالورونيك. بناء على نتائج الفحوصات السريرية وفحص السونار والنسجي المرضي فحوصات كيمياء النسيج المناعية

الكلمات المفتاحية: حامض الهيالورونيك ونخاع العظم وعيب الوتر والسونار و كيمياء النسيج المناعية.