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Histomorphological evaluation of low level laser therapy in dog achilles tendon injuries

Davood Sharifi[1*], Elias Vahed Dehkordi[2], Hamidreza Fattahian[2], Pejman Mortazavi[3], Saeed Hesaraki[3] and Mohammad Abedi[3]

[1]*Department of Veterinary Surgery, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
[2]*Department of Veterinary Surgery, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[3]*Department of Pathology, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran*

ABSTRACT

Objective of study is investigating the effect of low level laser on histomorphological changes in Achilles tendon in dogs. Twelve adult male dogs weighing 30 to 35 Kg (3-4 year old) were used for this study. Under general anesthesia and aseptic condition the dorsal surface of right Achilles tendon was exposed and after complete separation of connective tissue, it was splitted (striking 10 times) in full thickness in longitudinal fashion of 3cm in length in the mid-tendon area using BP blade no 15 in each one. These animals were divided into two groups of control (untreated) and experiment (treated with low level laser) having 6 animals each. The histomorphological changes was evaluated by using modified by collecting samples from mid splitted area of injured and normal tendons of each animal from treated and untreated. The results of this study indicated that additional exposure of tendon to low level laser as a local stimulator on severely injured or extensive lesion of Achilles tendon in dog will be highly useful in enhancing morphological changes in the treated tendon.

Key words: Histomorphological changes, Low level laser, Achilles tendon, Dog.

INTRODUCTION

Tendon healing is the main objective of the regenerative medicine. Although spontaneous healing can occur, this often results in the formation of scar tissue which is morphologically, biochemically and biomechanically different from healthy tendon tissue. Tendon injuries may require long term rehabilitation [1]. The Achilles tendon also known as the calcaneal tendon serves to attach the plantaris and gastrocnemius muscles to the calcaneus bone and is the strongest tendon in the musculoskeletal system. Its main function is in hind limb forward progression and it contributes to progressive support of the hock [2]. In general, the Achilles tendon injuries in dogs have resulted from massive trauma. In these types of injuries, depending on the trauma, the severity of the lesion may vary considerably, leading to stretching, partial lacerations or a complete rupture [3,4]. The main aim of reconstructive surgery or regenerative medicine for tendon injury is to restore an adequate morphological change to support body weight. Restoring function and use of the limb, which is the primary objective of tendon surgery or treatment in humans, is the instead a secondary objective in the dog [4]. However, the role of low level laser therapy in experimentally induced intense injury in the Achilles tendon has not been addressed yet in dogs. Therefore the present study was undertaken to evaluate the morphological changes after local application of low level laser on the healing process of this tendon in dogs and its final correlations between functional activity and clinical signs after surgery.

MATERIALS AND METHODS

All dogs used in the present study were cared for in accordance to the norms of the Islamic Azad University Faculty of Veterinary Medicine laboratory of animal experimentations; this investigation was approved by the Committee of Ethics in Research with Animals at the Islamic Azad University. Twelve adult male dogs weighing 30 to 35 Kg (3-4 year old) were used for this study. Anesthesia was induced using combinations of ketamine hydrochloride, acepromazine maleate and thiopental sodium (5mg/kg, IM, 0.1mg/kg IM, 10mg/kg, IV, respectively), and anesthesia was maintained using halothane 2%. After induction of anesthesia, the Achilles tendon of the right hind limb was exposed. Splitting of Achilles tendons in all dogs was done (striking 10 times) completely in full thickness in longitudinal fashion in about 3 cm in length in the mid-tendon area using Bard Parker blade no 15. Then, the surgical closure of the region was down as routine.

Animals were allocated randomly into two groups of 6 dogs each: control and experiment which were subdivided into two subgroups of one month and three months duration with 3 dogs each. Arsenate of Gallium Laser was utilized to treated animals in experimental group with the extension of wave of 860 nm, 10 mW power, 6 J/cm. The laser therapy was initiated on the post-surgical first day, with application once a day for 14 days and 10 minutes for each dog. No treatment was given to control group.

At the end of experimental period, dogs were submitted to euthanasia by an anesthetic overdoses and the tensile strength was measured by modified apparatus [5] by collecting full thickness samples (5 mm) from mid splitted tendon area of each animal from two groups. Statistical analyses were carried out using SPSS statistical software. Student t-test was employed to analyze two groups consecutively. Values of $P<0.05$ were considered as statistically significant.

RESULTS

There was no evidence of infection in the experimental group during laser treatments. Lameness degree showed by individual dog was approximately identical due to the resemblance of the damage. Lameness was most apparent during the first 3 post-surgical days and then gradually improved in the treated limb at the second week until the end of study compare to control group. Operative area swelling varied between individual dogs but it was less severe in the experimental dogs. Histomorphology changes, reduced inflammatory reactions and increasing of collagen synthesis were seen in both control and test groups after 3 months. The collagen fibers are more parallel in this area. In comparison, the connective tissue in test groups are more than in control groups after 30 days (Figs: 1, 2, 3). The connective tissue has more density in test groups after 90 days and collagen fibers are parallel and more similar to healthy tendon.

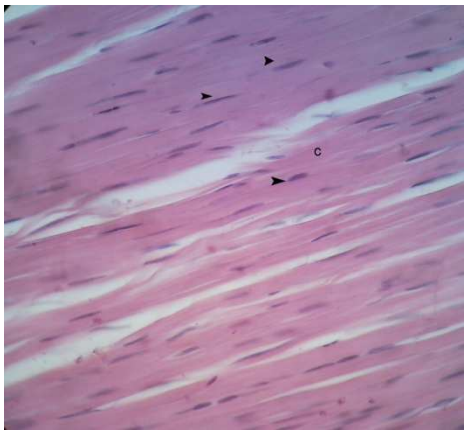

Figure1: Cross section from normal tendon with parallel and compact collagen fibers (c) with pointer showing nucleus of fibrocytes beside collagen fibers (H&E*140)

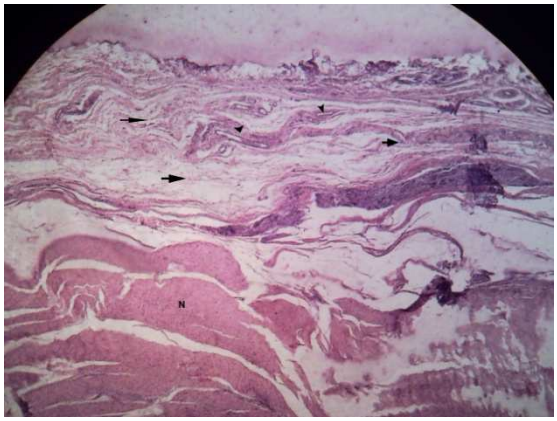

Figure 2: Control group after one month, Synthesis of connective tissue especially for fibers is light, in compression with treated group. Collage fibers (arrow) and revascularization (head of arrow) is obvious (H&E *64)

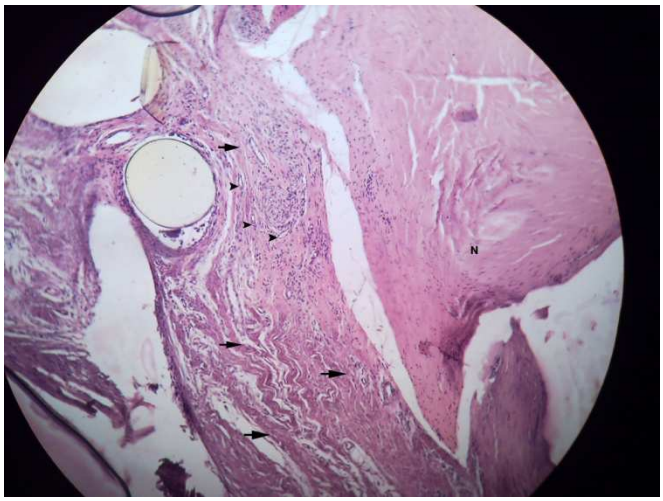

Figure 3: Treated group after one month. Production of connective tissue especially for collagen fibers is too much in compression with control group. Proliferation of dense connective tissue (arrow) with neovascularization (head of arrow) beside of intact tendon is obvious

DISCUSSION

Regeneration of damaged tendon tissue remains an underserved medical need for the field of orthopedics. The Achilles tendon is essential for maintain appropriate biomechanical function in ankle joint and particularly critical for activities. There are two concepts of surgical and non-surgical treatment for repair of tendon injuries. Current approaches to the surgical treatment of tendon injuries rely on fixation devices in combination with various suture techniques to re-attach tissues to the damaged area. Modern concepts of non–surgical treatment have been shown to produce good results with acceptable complication rates [5,6]. A successful tendon healing must have the appropriate strength, stability and tension to meet the dynamic workload. The healing process leads to a tissue functionally and structurally similar to the original tissue and collagenous connective is analogous to scar tissue that occurs in most parts of body [4,7,8].

The aim of this research was to define the effects of low level laser therapy on the severed Achilles tendon injury. Dogs in experiment group showed higher level of clinical consent and functional behavior on third weeks of treatment as compared to untreated group. The effects of low level laser therapy in experimental tendinopathy in the splitted area demonstrated the higher level of local reaction for speeding up healing as compared to untreated one [9-11]. The microscopic evaluation of samples indicated that there were positively significant differences between these groups but this was a very narrow difference within in the treated group. Injuries of the tendon are often accompanied by soft tissue or bone injuries; as a result, regeneration does not take place in an isolated environment. An important factor is whether a tendon heals without formation of adhesions to adjacent tissues, resulting in reduced gliding function [12]. As to diminish the local site effects and precipitate local stimulation as far as reposition of collagen fibers and early reorganization, local impaction of low level laser illustrated, it resolves inflammation, increase of the tenocytes proliferation and side by side restored tendon entirety correlated to clinical signs of having full limbs weight bearing [13]. The present study, in concert with previous ones, confirmed that

tendonitis repair due to increased fibroblastic/tenoblastic activity [14,15]. Cumulatively increased in mechanical property has direct relation in early maturation of fibroblasts and early parallel arrangement of collagen fibers and bundle formation.

The similar finding has been reported by Sharifi et al [13] and Gum Steven et al [16] concerning the effects of transcutaneous application of electrical stimulation on tensile strength in tendon of horse. The etiology of tendonitis are avascular changes, metabolic disturbances, degeneration, neural factors and neovascularization [17], but the acute swelling, matrix destruction and inflammation in tendon are similar to those seen in naturally occurring tendon injuries. Collagen production with a model of a precipitate rate of collagen turning over increased significantly and had effects on biochemical confidants of the tissue [1]. Previous reports showed a correlation exists among number of collagen fibrils and organization, size and mechanical strength [6,18]. The amount of regenerating tendons considered to be good indication for correlation between mechanical strength and the absolute amount of collagen being defined as biochemical thought assays for high density of collagen fibers in this study, Low level laser therapy shown to have beneficial effects when applied to tendon healing. Collagen production with a model of an accelerated rate of collagen turnover increased significantly in the treated tendon. It had a direct effect on biochemical properties of the tissue. The results of Sharifi et al [13] indicated a correlation among collagen fibril number and organization, size and biochemical strength. These previous studies relied on the collagen profiles of mature tendons, whereas in our study, the amount of regenerating tendons as the correlation among histomorphological changes, biomechanical strength and the total collagen as defined biochemical thought assays for density of connective tissues. The results of the present study support the view that direct application of low level laser on severely injured tendons will be highly useful in enhancing collagen fibers in the treated tendon. The results of this study showed that application of local impaction of low level laser fibers into extensive lesion of tendon will be useful and enhance amount of connective tissues in the treated tendon. This research strongly suggests using direct marker of the effect of local use of collagen content in injured tendon. Histomorphology changes, reduced inflammatory reactions and increasing of collagen synthesis were seen in both control and test groups after 3 months. The collagen fibers are more parallel in this area. In comparison, the connective tissue in test groups are more than in control groups after 30 days. The connective tissue has more density in test groups after 90 days and collagen fibers are parallel and more similar to healthy tendon.

CONCLUSION

The results of this study indicated that additional exposure of tendon to low level laser as a local stimulator on severely injured or extensive lesion of Achilles tendon in dog will be highly useful in enhancing morphological changes in the treated tendon.

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