ORIGINAL ARTICLE

Nr 2023;13 (1):11-17

Perfecting Achilles Tendinopathy Inducement: Preliminary Study in Animal Model - BONAR Score as Quantitative Indicator

Henry Ricardo Handoyo[1], Mohamad Hidayat[2], Teguh Wahju Sardjono[3], Setyawati Soeharto[4], Shod Abdurrachman Dzulkarnain[1]

[1] *Doctoral Program in Medical Sciences, Faculty of Medicine, Brawijaya University, Malang, Indonesia*
[2] *Department of Orthopedic and Traumatology of Saiful Anwar General Hospital, Faculty of Medicine, Brawijaya University, Malang, Indonesia*
[3] *Department of Clinical Parasitology of Saiful Anwar General Hospital, Faculty of Medicine, Brawijaya University, Malang, Indonesia*
[4] *Department of Clinical Pharmacology of Faculty of Medicine, Brawijaya University, Malang, Indonesia*

CORRESPONDING AUTHOR:
Henry Ricardo Handoyo
Doctoral Program in Medical Sciences
Faculty of Medicine
Brawijaya University, Malang
Jl. Veteran Malang 65145
East Java, Indonesia
E-mail: henryricardohandoyo@gmail.com

DOI:
10.32098/mltj.01.2023.02

LEVEL OF EVIDENCE: N/A

SUMMARY

Background. As a common ailment suffered by runners and athletes, Achilles tendinopathy pathogenesis is not well understood. To reveal its cellular and molecular changes, universal and standardized method to induce Achilles tendinopathy in animal model (*in vivo*) study should be established. There are 2 major guidelines to induce Achilles tendinopathy: run the rats on treadmill without inclination and run the rats on treadmill with 10° inclination.

Objective. To compare available Achilles tendinopathy induction procedure hence determining the most effective procedure.

Methods. Laboratory experimental animal study (*in vivo*) with post-test only control group approach. 2 procedures to induce Achilles tendinopathy on Wistar rats were performed: running at 15 m/min for 180 minutes, and running at 25 m/min for 15 minutes with 10° inclination for 60 minutes. Histological changes of Achilles tendon were converted into Bonar score while several inflammatory markers such as extracellular high mobility group box-1 (HMGB-1), tumor necrosis factor-alpha (TNF-α), C-X-C motif chemokine ligand-12 (CXCL-12), and macrophage-2 (M2) were measured.

Results. Highest Bonar score was obtained from rats that run at 25 m/min for 15 minutes with 10° inclination for 60 minutes. Highest level of extracellular HMGB-1, TNF-α, and CXCL-12 was also obtained from similar group. This group also showed widest M2 coverage area.

Conclusions. Running the rats at 25 m/min for 15 minutes with 10° inclination for 60 minutes is more effective than running the rats at 15 m/min for 180 minutes without inclination to induce Achilles tendinopathy.

KEY WORDS

Achilles tendinopathy; animal model; Bonar score; inflammatory response; treadmill.

INTRODUCTION

Achilles tendinopathy is a pathological condition frequently found in adult with history of overused ankle. About 30% of professional runners suffered Achilles tendinopathy, while its incidence varies between 7-9% (1). Achilles tendon is the most frequent tendon suffering tendinopathy due to competitive or recreational sports. Usually, damage occurs 2-7 cm above calcaneal insertion (2). Achilles tendinopathy can become permanent ailment and inhibit daily activities or athletic carrier. Achilles tendinopathy happens to 37/100,000 European population,

including young adult (37-44 years old) (2). Achilles tendinopathy manifest as chronic activity related pain, tenderness, localized edema, and decrease dorsiflexion of the ankle. But these symptoms are not always present in all case. Certain study performed in Tor Vergata University Hospital at 2018 showed that the majority of population who suffered Achilles tendinopathy did not present any symptoms related to Achilles tendon dysfunction (3). Most cases were detected after thorough evaluation of ankle radiographs.

Extrinsic factors related to Achilles tendinopathy included physical exercise, repetitive loading, improper workout technique, shoes, and uneven running track (4). Intrinsic factors related to Achilles tendinopathy included elderly age, anatomical variation of leg, leg length discrepancy, joint laxity, muscle weakness, overweight, and systemic abnormalities (5). Overused Achilles tendon caused microtrauma which induces collagen degradation and tenocytes transformation, both induced inflammatory responses.

As mentioned before, systemic abnormalities and overweight is one of the risk factors of Achilles tendinopathy. It has been identified that Achilles tendinopathy is common among patient with obesity or diabetes mellitus, either type 1 or type 2 (2, 6, 7). Obesity increases mechanical loading on Achilles tendon especially during running. Higher mechanical loading means higher catabolism of collagen fiber and extracellular matrix. In people with obesity, the catabolism of collagen fiber and extracellular matrix is too high to be compensated by normal anabolism. Higher rate of proteoglycans catabolism leads to swelling due to water retention and increases metalloproteinases expression. It also releases inflammatory molecules such as inteleukin-1β. Previous study performed in Italy in 2012 showed 23.8% people with obesity suffered Achilles tendinopathy (P-value = 0.007) (6).

In patients with diabetes mellitus, hyperglycemia reduces tendon homeostasis and induces vascular hyperplasia, disorganized collagen and altered viscoelastic properties (7). Previous study performed at several orthopedic center in Italy during 2021 (2) showed 28% patients with diabetes mellitus, either type 1 or type 2, suffered Achilles tendinopathy.

Based on several information mentioned before, we can assume that inflammation is the origin of Achilles tendinopathy. Recurrent exposure to inflammatory response due to tendon overuse induces degenerative change in the tissue of Achilles tendon. Tendon overuse induces microtrauma hence releases cytokine and chemokine. Microtrauma also degrades collagen matrix, stimulates neovascular formation, and triggers apoptosis of tendon tenocytes. During its apoptosis, tenocyte release high mobility group box 1 (HMGB-1) protein as damage-associated molecular patterns (DAMPs) (8). HMGB-1 induces *toll-like reseptor-4* (TLR-4) on the surface macrophage and other cells to produce inflammatory cytokine and chemokine using NF-ϰB pathway. There are 2 pathways of NF-ϰB: canonical pathway which produces inflammatory cytokine especially tumor necrosis factor-alpha (TNF-α) and non-canonical pathway which produce several chemokines such as C-X-C motif chemokine ligand 12 (CXCL-12) which attracts cellular components of immune response and maintains inflammation.

Prolonged inflammatory response alters the tissue morphology of Achilles tendon. Prolonged inflammation, including over expression of macrophage 2 (M2) disturbs physiological healing process, inducing degenerative changed (9). The tendon is noticeably thicker and stiffer, collagen fiber was disorganized, cells nuclei were more ovoid, hypercellularity of muscle cell and tenocyte was noted, increased neovascularization can be observed, and ground substance such as elastin was noticeably denser (9, 10). This transformation could be measured using Bonar score, universal standard for tissue transformation in Achilles tendinopathy. Prolonged inflammatory response also increases macrophage 2 differentiation and infiltration in the tendon. It repeatedly heals the tendon but also prolongs the inflammation (11).

To understand Achilles tendinopathy and produce proper therapy for it, study on animal model is required. But standardized method to induce Achille tendinopathy on animal model has not been settled yet. Some methods like collagenase injection or microincision induce sudden profound inflammatory response on the tendon, hence not represent the actual gradual process of Achilles tendinopathy. The most acceptable method was to run the animal model on a treadmill with certain velocity and duration. But there is diverse velocity and duration used in several studies. In certain study, the animal shall run at 15 m/minute for 180 minutes (12), while on the other study the animal shall run at 25 m/minute for 60 minutes (13). Other study applied inclination on the treadmill (14, 15), while the others not. But among several methods proposed, none has been declared as the most effective.

This preliminary study aims to evaluate prior Achilles tendinopathy induction methods to determine the most effective method. Bonar score will be used as prominent indicator while other molecular variable support inflammatory evidence.

METHODS

Animal model preparation and tendinopathy induction

Male Wistar rats (*Rattus norvegicus*), 2 months old, 250 grams, were chosen as animal model. The rats were obtained from Bioscience Laboratory, Brawijaya University, Indonesia, and adjusted in new environment for 2 weeks. Ethical clearance was approved by Health Research Ethics Committee Faculty of Medicine, Brawijaya University (23/EC/KEPK-S3/02/2022

– Date of approval: 04 February, 2022) and the methods were carried out in accordance with the approved guidelines.
To induce tendinopathy on Achilles tendon, the rats were made to run on treadmill. 27 rats were divided into 3 groups: (i) negative control group, 9 rats without any intervention; (ii) 9 rats that run at 15 m/min for 15 minutes on the first 7 days, then increased to 15 m/min for 180 minutes on weekdays (5 days) for 3 weeks; and (iii) 9 rats that run at 25 m/min for 15 minutes with 10° inclination on the first 7 days, then increased to 25 m/min for 60 minutes on weekdays (5 days) for 3 weeks. After 4 weeks of tendinopathy induction procedure, the rats were sacrificed using ketamine injection. The right Achilles tendon was submerged in phosphate buffer saline for cross-section area measurement and ELISA procedure while the left Achilles tendon was submerged in formalin 10% solution for histopathology slide preparation.

Cross-section area of Achilles tendon measurement

After the rats were sacrificed, the right Achilles tendon perpendicular axis lengths were measured in proximal region, mid region, and distal region. Products of each perpendicular length mark the area of respective region. CSA is the mean of these 3 regions.

Evaluating Bonar score

Hematoxylin-eosin staining was performed on histopathology slide to facilitate tissue observation. Observation was performed under 200 times magnification. Previous publication (10) was used as guidance for Bonar score. If the score is more than 11.6, Achilles tendinopathy could be assumed.

Extracellular HMGB-1, TNF-α, and CXCL-12 levels measurement

As described in previous publication (12), right Achilles tendon in PBS was cooled in 4 °C for 24 hours. Then the tendon was homogenized in protein extractor solution (Cat No. 17081) as mentioned in its protocol. ELISA kit and ELISA reader was used to measure the extracellular HMGB-1, TNF-α, and CXCL-12 levels. ELISA kit Cat. No Cat No. E0257Ra was used to measure extracellular HMGB-1 levels. ELISA kit Cat.No. E0764Ra was used to measure TNF-α levels. ELISA kit Cat.No. E1538Ra was used to measure CXCL-12 levels.

Evaluating M2 differentiation

Immunohistochemical staining was performed to detect macrophage 2 differentiation. Antibody CD-163 (Cat. No. sc-58965) was chosen as macrophage 2 marker (16). Immunohistochemical staining system (N-Histofine Simple Stain max PO (M), code: 414131F) was used to stain antibody-marked tissue and cell. Observation was performed under 400 times magnification.

Statistical analysis

Each variable was tested statistically using one-way ANOVA when the data was normally distributed and homogenous. When the data was not normally distributed or homogenous, it was tested using Kruskal-Wallis.

RESULTS

Cross-section area of Achilles tendon after tendinopathy induction

This study showed higher cross-section area on running rats compared to control (**figure 1**). Highest cross-section area was found in group (iii), 5.0 mm^2. Statistical analysis showed no significant difference among the groups, proved by P-value = 0.273.

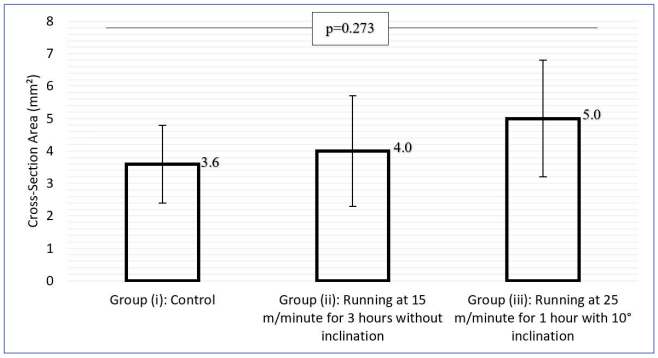

Figure 1. Cross-section area of Achilles tendon from each group.

Bonar score of Achilles tendon after tendinopathy induction

This study showed higher Bonar score on running rats compared to control (**figure 2**). Highest Bonar score was found in group (iii), 12.6 (**figure 3**). Statistical analysis showed significant difference among the groups, proved by P-value < 0.001. *Post-hoc* analysis shows significant difference between group (ii) and group (iii), proved by P-value = 0.008.

Extracellular HMGB-1 level of Achilles tendon after tendinopathy induction

This study showed no difference between group (i) and group (ii), with HMGB-1 levels 1.2 ng/L. The HMGB-1 level in group (iii) is 1.9 ng/L (**figure 4**). Statistical analysis showed significant difference among the groups, proved by P-value < 0.001. *Post-hoc* analysis shows significant difference between group (ii) and group (iii), proved by P-value = 0.003.

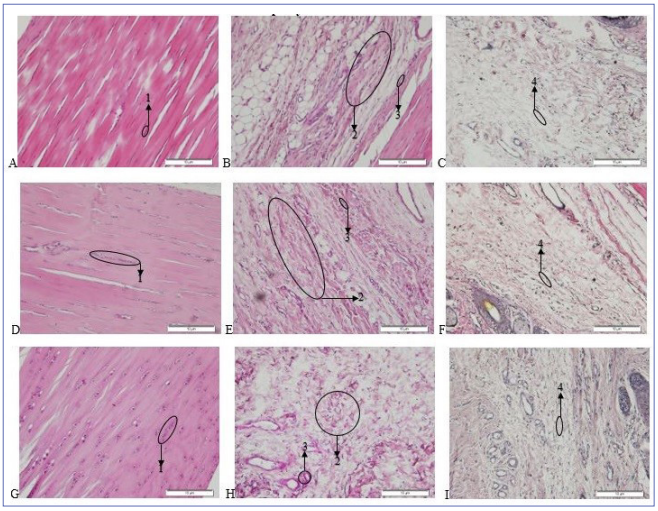

Figure 2. Histopathology observation of rats Achilles tendon; 1: nucleus, 2: collagen, 3: vascular; 4: ground substance (black).

(A-C) come from group (i); **(D-F)** come from group (ii); **(G-I)** come from group (iii).

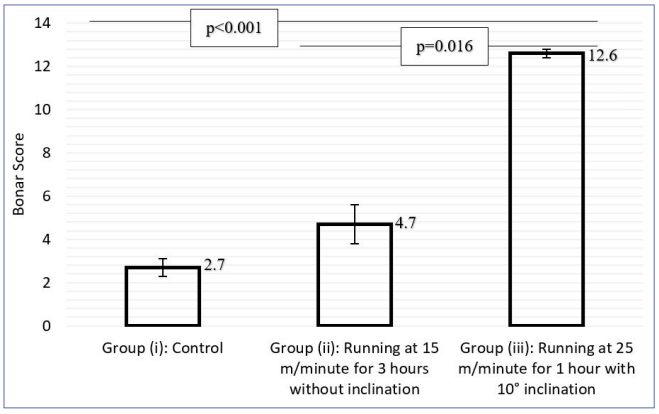

Figure 3. Bonar score from each group.

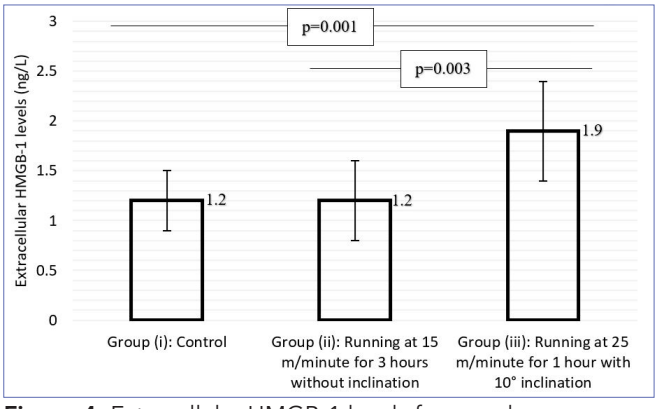

Figure 4. Extracellular HMGB-1 levels from each group.

TNF-α level of Achilles tendon after tendinopathy induction

This study showed higher TNF-α levels in running rats compared to control rats. Highest TNF-α levels found in group (iii), 77.4 ng/L (**figure 5**). Statistical analysis showed significant difference among the groups, proved by P-value < 0.002. *Post-hoc* analysis shows significant difference between group (ii) and group (iii), proved by P-value = 0.010.

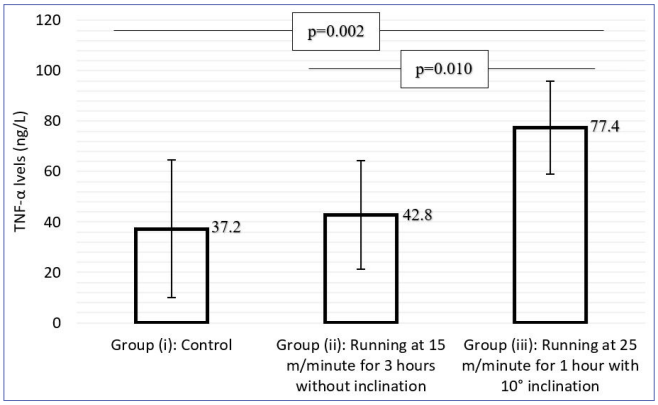

Figure 5. TNF-α levels from each group.

CXCL-12 level of Achilles tendon after tendinopathy induction

This study showed higher CXCL-12 levels in running rats compared to control rats. Highest CXCL-12 levels found in group (iii), 109.4 ng/L (**figure 6**). Statistical analysis showed significant difference among the groups, proved by P-value < 0.017. *Post-hoc* analysis shows significant difference between group (ii) and group (iii), proved by P-value = 0.048.

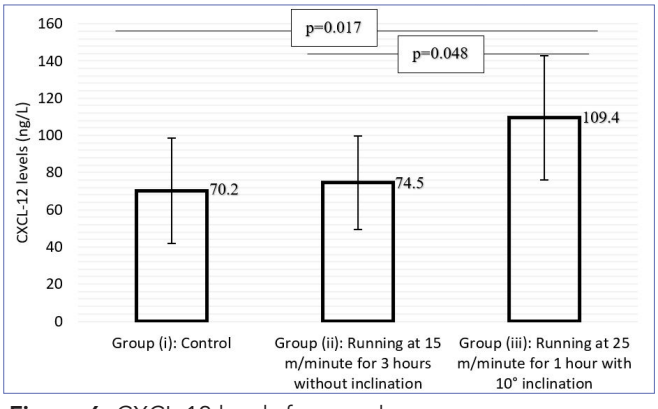

Figure 6. CXCL-12 levels from each group.

M2 differentiation of Achilles tendon after tendinopathy induction

This study showed wider M2 covered area in running rats compared to control rats (**figure 7)**. Widest M2 covered area was found in group (iii), 15.7% (**figure 8)**. Statistical analysis showed significant difference among the groups, proved by P-value < 0.001. *Post-hoc* analysis shows significant difference between group (ii) and group (iii), proved by P-value = 0.008.

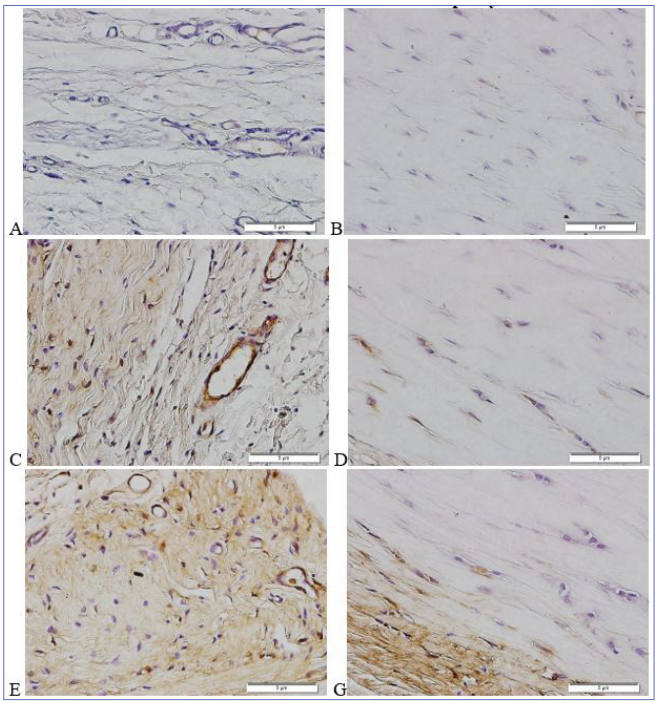

Figure 7. Immunohistochemical staining of rats Achilles tendon, brown color shows M2 coverage area.

(A, B) come from group (i); **(C, D)** come from group (ii); **(E, F)** come from group (iii).

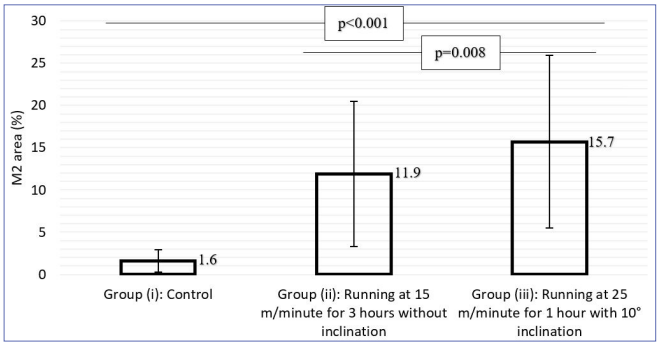

Figure 8. M2 covered area from each group.

DISCUSSION

As one of the most frequent diseases suffered by athletes, Achilles tendinopathy's pathogenesis was not well understood. It has been concluded that tendons overuse is the main source of Achilles tendinopathy, but the molecular and cellular aspect of Achilles tendinopathy has not been defined thoroughly. There are several changes occurred such as increasing inflammatory immune responses, collagen fiber degradation, and cellular transformation (10, 17). Dysregulation of matrix metalloproteinase 7 and matrix metalloproteinase 3 also has been proposed as pathogenesis of Achilles tendinopathy (18). Some study also mentioned transformation of tenocyte in Achilles tendinopathy where the tenocyte tends be to more ovoid, produces more proteoglycans, and seems like chondrocyte (18). Therefore, more advanced and deeper study must perform especially *in vivo*. *In vivo* study enables easier tissue observation and protein measurement compared to clinical research because it does not require patient's agreement.

Bonar score has become standardized criteria to evaluate microanatomy changes in Achilles tendon. As mentioned in previous study, Bonar score represents pathological tissue transformation due to tendon overuse or tissue damaged including abnormal cellular morphology, collagen disarrangement, hypercellularity, increasing vascularity, and ground substance appearance (10). Higher Bonar score indicates worse damage on tendons tissue.

But there are so many protocols performed to induce Achilles tendinopathy on animal model. Most procedure performed by running the animal model, usually rats, on the treadmill, but some applied certain inclination on the treadmill (14, 15) while the others did not (12). The result of these studies is varied. Although not using Bonar score as primary indicator, a study performed in Pittsburgh University (12), where the rats run on horizontal treadmill machine at 15 meters/minute velocity for 3 weeks (50 minutes each day) without inclination, showed greater inflammatory response on Achilles tendon of running rats compared to control group, proven by higher concentration of prostaglandin-2. On another study, performed in University of British Columbia (15), where the rats were made to run in uphill treadmill machine (10° inclination) at 1 kilometer/hour velocity for 12 weeks (60 minutes each day), showed significant tissue degradation on running rats compared to control group, proven by more conspicuous tenocyte and degrading collagen fibers in running rats. Other study performed in Indiana University (14), also applied certain inclination on the treadmill. The intervened rats were run on treadmill with 15° inclination, 25 meters/minutes velocity for an hour, 5 days a week during 9 weeks observation period. Interestingly there is no significant differences among their tendon tissue compared to control group.

Another popular method to induce Achilles tendinopathy on animal model is collagenase injection directly into the animal

ankle. This method is very preferable because tendon degradation of the animal model can be observed in short period. On study performed at Shantou University Medical College (19), collagenase injection on rats Achilles tendon resulted in stiffer collagen fiber and degrading tissue of Achilles tendon compared to control group. Similar results can be found in certain study performed in IRCCS Galeazzi Orthopedic Institute (20) where disorganized collagen matrix and increased number of rounded cell could be found on collagenase injected rats.

However, our study did not adopt collagenase injection methods out of actual Achilles tendinopathy pathogenesis consideration. Achilles tendinopathy is caused by gradual overuse of Achilles tendon during running instead of sudden degradation of matrix due to collagenase. We preferred to simulate actual pathogenesis of Achilles tendinopathy by running the rats on the treadmill.

Our study showed higher Bonar score in the group of rats that run on the treadmill with 10° inclination which indicates significant tissue transformation in the inclination-group. Compared to our study, previously mentioned study performed in Indiana University, Indianapolis (14), showed different results. Our study, instead, showed more similar results with study performed in University of British Columbia (15). Like our study, several changes observed in those studies were nucleus morphology, collagen arrangement, and tenocyte cytoplasm.

Our study also showed wider cross-section area (CSA) of running rats compared to control group. As mentioned in previous systematic review published at 2022 (21), tendon connective tissue involvement plays important role in Achilles tendinopathy pathogenesis. Wider CSA is formed as an adaptation towards repetitive overload. Normally repetitive overload induced increasing tenocyte and collagen fiber of tendon. But if the repetitive overload was excessive, collagen fiber formation would not be unable to keep up with tenocyte production and producing stiffer and wider tendon (21).

Running the rats on the treadmill as an attempt to induce Achilles tendinopathy also increases several inflammatory markers. As previously mentioned, study performed at Mechano-Biology Laboratory of University of Pittsburg, Pennsylvania (7), showed increasing level of HMGB-1 and prostaglandin E-2 (PGE-2). It also showed increasing level of matrix metalloproteinase-3, important matrix-degrading enzyme. Our study also showed increasing levels of HMGB-1, especially in the inclination-group (iii). But, instead of measuring PGE-2, which directly involves in inflammatory response, our study measures the level of TNF-α and CXCL-12 which induces and enhances PGE-2 synthesis respectively. Our study showed increasing level of TNF-α and CXCL-12, proving inflammatory response in running rats. Increasing TNF-α and CXCL-12 also prove NF-ϰB pathway involvement either the classical pathway and non-classical pathway, respectively. In accordance with our results, previous study performed in University of Salemo, using tendinopathy induced human tendon tissue (18), also showed increasing inflammatory marker such as TNF-α, IL-1β, and IL-6. Interestingly, TGF-β (tumor growth factor β) also increased in this model, indicating tissue recovery. But the recovery could not be performed optimally, hence TGF-β induced tendon deformity such as pathologically thickening and stiffness.

Interesting comparison and greater insight can be found from study performed in Liege University, Belgium (22). In this study the rats were made to run on treadmill with 17 meters/minutes velocity for 1 hours, 3 days a week during 5 weeks observation period. Magnificently, this study compared the results from 3 different group: U group or negative control, C group which the rats run on uphill track with 15° inclination, and E group which the rats run on downhill track which 15° declination. That study showed worse condition of Achilles tendon tissue during hematoxylin-eosin slide observation obtained from the rats from E group. The rats which run downhill have more neovascularization and larger CSA compared to the ones who run uphill. Macrophage observation and inflammatory markers for similar method (running downhill), as in that study, will be our top priority for further research.

CONCLUSIONS

This study proved that running at 25 m/minute for 1 hour with 10° inclination was the most effective tested method to induce Achilles tendinopathy on rats as animal model. Highest Bonar score obtained from rats which run 25 m/minute for 1 hour with 10° inclination for 1 hour or group (iii). Inflammatory markers also showed highest levels on the aforementioned group. Hence this method can be considered as preferred method for inducing Achilles tendinopathy.

FUNDINGS

None.

DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

HRH: funding, conceptualization, data collection and analysis, manuscript writing, literature revision and analysis. MH, TWS, SS: study supervision, manuscript revision and reviewing. SAD: data collection research.

ACKNOWLEDGEMENTS

The authors thank to support staff from Bioscience Laboratory of Brawijaya University.

CONFLICT OF INTERESTS

The author declare that they have no conflict of interests.

REFERENCES

1. Ackermann PW, Renström P. Tendinopathy in sport. Sports Health. 2012;4(3):193-201. doi: 10.1177/1941738112440957.
2. Oliva F, Marsilio E, Asparago G, et al. Achilles Tendon Rupture and Dysmetabolic Diseases: A Multicentric, Epidemiologic Study. J Clin Med. 2022;11(13):3698. doi: 10.3390/jcm11133698.
3. Via AG, Oliva F, Padulo J, Oliva G, Maffulli N. Insertional Calcific Tendinopathy of the Achilles Tendon and Dysmetabolic Diseases: An Epidemiological Survey. Clin J Sport Med. 2022;32(1):E68-73. doi: 10.1097/JSM.0000000000000881.
4. Aicale R, Oliviero A, Maffulli N. Management of Achilles and patellar tendinopathy: What we know, what we can do. J Foot Ankle Res. 2020;13(1):59. doi: 10.1186/s13047-020-00418-8.
5. Darrieutort-Laffite C, Soslowsky LJ, Goff B Le. Molecular and structural effects of percutaneous interventions in chronic achilles tendinopathy. Int J Mol Sci. 2020;21(19):7000. doi: 10.3390/ijms21197000.
6. Abate M, Oliva F, Schiavone C, Salini V. Achilles tendinopathy in amateur runners: Role of adiposity (Tendinopathies and obesity). Muscles Ligaments Tendons J. 2012;2(1):44-8. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/23738273/.
7. Nobre de Lacerda Brito AC, Alves Martins W, da Silva Queiroz PC, et al. Combined treadmill running and insulin therapy favors the stabilization of glycemic metabolic parameters and avoids increased achilles tendon rigidity in diabetic rats. Muscles Ligaments Tendons J. 2020;10(3):442-50. doi: 10.32098/mltj.03.2020.14.
8. Dakin SG, Newton J, Martinez FO, Hedley R, Gwilym S, Jones N, et al. Chronic inflammation is a feature of Achilles tendinopathy and rupture. Br J Sports Med. 2018;52(6):359-67. doi: 10.1136/bjsports-2017-098161.
9. Li HY, Hua YH. Achilles Tendinopathy: Current Concepts about the Basic Science and Clinical Treatments. Biomed Res Int. 2016;2016:6492597. doi: 10.1155/2016/6492597.
10. Fearon A, Dahlstrom JE, Twin J, Cook J, Scott A. The Bonar score revisited: Region of evaluation significantly influences the standardized assessment of tendon degeneration. J Sci Med Sport. 2014;17(4):346-50. doi: 10.1016/j.jsams.2013.07.008.
11. Parisi L, Gini E, Baci D, et al. Macrophage Polarization in Chronic Inflammatory Diseases: Killers or Builders? J Immunol Res. 2018;2018:8917804. doi: 10.1155/2018/8917804.
12. Zhao G, Zhang J, Nie D, et al. HMGB1 mediates the development of tendinopathy due to mechanical overloading. PLoS One. 2019;14(9):e0222369. doi: 10.1371/journal.pone.0222369.
13. Rios JL, Boldt KR, Mather JW, Seerattan RA, Hart DA, Herzog W. Quantifying the Effects of Different Treadmill Training Speeds and Durations on the Health of Rat Knee Joints. Sport Med - Open. 2018;4(1):15. doi: 10.1186/s40798-018-0127-2.
14. Dirks RC, Richard JS, Fearon AM, et al. Uphill treadmill running does not induce histopathological changes in the rat Achilles tendon. BMC Musculoskelet Disord. 2013;14:90. doi: 10.1186/1471-2474-14-90.
15. Abraham T, Fong G, Scott A. Second harmonic generation analysis of early Achilles tendinosis in response to in vivo mechanical loading. BMC Musculoskelet Disord. 2011;12:26. doi: 10.1186/1471-2474-12-26.
16. Hu JM, Liu K, Liu JH, Jiang XL, Wang XL, Chen YZ, et al. CD163 as a marker of M2 macrophage, contribute to predicte aggressiveness and prognosis of Kazakh esophageal squamous cell carcinoma. Oncotarget. 2017;8(13):21526-38. doi: 10.18632/oncotarget.15630.
17. Abate M, Silbernagel KG, Siljeholm C, et al. Pathogenesis of tendinopathies: inflammation or degeneration? Arthritis Res Ther. 2009;11(3):235. doi: 10.1186/ar2723.
18. Ciardulli MC, Scala P, Giudice V, et al. Stem Cells from Healthy and Tendinopathic Human Tendons: Morphology, Collagen and Cytokines Expression and Their Response to T3 Thyroid Hormone. Cells. 2022;11(16):2545. doi: 10.3390/cells11162545.
19. Wu Y, Qian J, Li K, Li W, Yin W, Jiang H. Farrerol alleviates collagenase-induced tendinopathy by inhibiting ferroptosis in rats. J Cell Mol Med. 2022;26(12):3483-94. doi: 10.1111/jcmm.17388.
20. Orfei CP, Lovati AB, Viganò M, Stanco D, Bottagisio M, Di Giancamillo A, et al. Dose-related and time-dependent development of collagenase-induced tendinopathy in rats. PLoS One. 2016;11(8):e0161590. doi: 10.1371/journal.pone.0161590.
21. Rubio-Peirotén A, García-Pinillos F, Cartón-Llorente A, Jaén-Carrillo D, Abat F, Roche-Seruendo LE. Lower-Limb Connective Tissue Morphologic Characteristics in Runners. How Do They Relate with Running Biomechanics and Tendon Pathology? A Systematic Review. Muscles Ligaments Tendons J. 2022;12(3):331-51. doi: 10.32098/mltj.03.2022.09.
22. Kaux JF, Drion P, Libertiaux V, Colige A, Hoffmann A, Nusgens B, et al. Eccentric training improves tendon biomechanical properties: A rat model. J Orthop Res. 2013;31(1):119-24. doi: 10.1002/jor.22202.