Research Article

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Investigating the Effects of Dry Needling on Hamstring Muscles Flexibility: A Randomized Controlled Trial

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ABSTRACT

Introduction: Flexibility or the ability of a muscle to increase in length is an integral part of musculoskeletal characteristics and is essential in preventing musculoskeletal injuries and increasing functional levels. Hamstring muscles rupture is a common injury. One of the important factors in the occurrence of this injury is poor hamstring muscle flexibility, which because of its stiffened structure, has less ability to quickly increase in length. Some researchers have recently suggested that the dry needling technique could reduce the number of treatment sessions for hamstring muscle tightness and bring faster and more effective results. Accordingly, this study aims to investigate the effect of the application of dry needling through an acupuncture technique on hamstring muscle flexibility.

Materials and Methods: The present study is a single-blinded randomized controlled trial in which 16 individuals with bilateral hamstring muscle shortness were randomly allocated into dry-needling and sham-needling groups. The sampling method was non-probability convenience. The outcome measures were the right and left active knee extension range of motion, assessed before and after the first, third, and fifth sessions of intervention. A 2-factor mixed analysis of variance was applied to determine the differences between and within the two groups.

Results: Except for the group main effect, time main effect and interaction effect were statistically significant for the right and left active knee extension range of motion (P<0.001). There were differences in behavioral patterns in groups; accordingly, in the real dry-needling group, a significant difference was detected across times. However, in the sham dry-needling group, no significant difference was observed.

Keywords:

Dry needling; Hamstring muscles tightness; Flexibility; Range of motion; Knee joint **Conclusion:** The application of dry needling into motor points of hamstring muscles in individuals with bilateral hamstring muscles shortness could improve flexibility and increase the range of knee extension after 3 and 5 sessions of intervention because of the therapeutic effects of the dry needling technique in improving the muscle flexibility.

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1. Introduction

uscle flexibility or the ability to increase length is an integral part of musculoskeletal characteristics and is important in preventing musculoskeletal injuries along with increasing functional levels

[1]. Hamstring muscles rupture is a common injury that accounts for about 53% of ruptures of the biceps femoris. One of the most important causes of this injury is poor hamstring muscle flexibility as stiffened muscle is less able to quickly increase in length [2]. Decreased hamstring muscle flexibility is associated with an increased risk of lower limb injuries [3]. It may also be associated with changes in the curvature of the spine in the sagittal plane, which increases the risk of spinal cord injury because of mechanical stress [4]. Moreover, studies have shown that hamstring muscle tightness leads to intermuscular imbalance, muscle injuries, and patellofemoral pain, and accelerates the process of low back pain. There are various treatments to increase the length of the hamstring muscles, namely static stretching, dynamic stretching, proprioceptive neuromuscular facilitation, muscle energy technique, along with techniques, such as knee extension and straight leg raise. However, these treatments often last up to about 10 weeks [5].

However, one of the treatments that has recently attracted attention for the treatment of tight hamstring muscles is the use of the dry needling technique. The dry needling technique is an intervention during which a thin needle is inserted through the skin into the tissue. There are some related mechanisms by which dry needling could increase muscle length. Applying the dry needling technique to shortened sarcomeres creates a local tension in the contracted cytoskeletal structures, separating the myosin fibers from the titanium gel site (a spring-like molecule that holds myosin in its molecular position) in the Z-band. This can allow the sarcomere to regain its resting length by reducing the overlap angle between actin and myosin filaments [6, 7]. Also, in shortened muscles, the blood vessels are compressed and this localized blood pressure (ischemia) prevents the supply of energy, and the amount of oxygen is reduced. Following dry needling, the muscle can be relaxed by separating the actin from the myosin, thereby breaking the cycle of energy depletion [8]. Moreover, by applying the dry needling technique, changes are made in the blood flow of the muscle, which is reduced by shortening, and vasodilation and increased blood flow occurs in the tight hamstring muscles [9]. The main cause of increased blood flow is the release of vasodilators, such as calcitonin gene-related peptides and substances following the stimulation of C and A δ fibers by an axonal reflex that causes small vessels to open and increase the blood flow [10].

Dry needling can also increase the level of oxygen at the site of stimulation by increasing the proteins that respond to hypoxia. These proteins cause blood vessels to grow and dilate and alter glucose metabolism in oxygendeficient tissues. Accordingly, they potentially increase oxygen and capillaries and improve blood circulation in muscles [10]. Dry needling can activate signal-related extracellular kinases and signaling pathways related to focal adhesion of the kinase. It also activates nicotinamide adenine dinucleotide phosphate, which improves energy metabolism in muscles and allows muscles to regain their normal length [11].

Moreover, there is evidence that needling a muscle could change the electromyographic activity of the stimulated muscle; therefore, it can change muscle tension and allow the muscle to relax and lengthen [12]. Some researchers have recently suggested that this technique could reduce the number of treatment sessions for hamstring muscle tightness and bring faster and more effective results. Therefore, it has more economic benefits in terms of health [9, 10].

Accordingly, Alaei et al. investigated the immediate effect of dry needling on hamstring muscle flexibility in individuals with hamstring muscles shortness. In this study, dry needling was applied to the motor points of the hamstring muscles with the fast in-and-out technique by inserting and removing the needle for 1 min. The knee range of motion (ROM), muscle compliance, passive peak torque, and stretch tolerance were measured before, immediately after, and 15 min after dry needling. The results showed an increase in knee ROM along with other outcome measures which was better in dry needling compared to the sham group [13]. Moreover, Mason et al. examined the effectiveness of dry needling and stretching versus stretching alone on hamstring muscle flexibility in patients with knee pain. In this study, patients with hamstring muscle shortening were randomly divided into 2 groups of dry needling and sham-needling with hamstring muscles stretch. In the dry-needling group, the needle was applied at the trigger points of hamstring muscles by piston method, and in the shamneedling group, it was applied by squeezing a plastic tube in non-trigger points and the patients received the treatment technique in 2 sessions with a 3-day interval. The assessments were performed immediately after the application of the technique, one day, three days, and seven days after the intervention. The results showed significant improvements in the ROM of the knee and a reduction in pain in the dry needle group [14].

Moreover, Sadat et al. examined the effect of dry needling on flexibility and electrophysiological indices in healthy men with short hamstring muscles. The dry needling was applied to the motor points of the hamstring muscles by inserting and removing the needle for 1 min. The assessments were performed before, immediately after, and 1 week after the intervention and consisted of knee flexibility with passive knee extension test, H reflex latency, and neuronal motor excitability. The results showed no significant differences between the 2 groups in any of the variables [15].

To the best of our knowledge, there is limited research investigating the effect of dry needling on muscle flexibility. Also, in these studies, dry needling has been applied in the form of a fast in-and-out technique. Moreover, treatment sessions had been limited to one session. Hence, the current study has been conducted to investigate the effects of applying dry needling through an acupuncture technique (reduction technique) on hamstring muscle flexibility in 5 sessions.

From the point of view of Chinese medicine, needling in acupuncture points makes changes in Qi energy. When a dysfunction, such as over-activity occurs, for instance in muscle stiffness and shortness, extra energy is placed in the desired location. Needling in acupuncture points could reduce energy. After applying the needle and moving it counterclockwise, the Qi energy is moved and reduced [16, 17]. This method also increases the person's tolerance to stretch by affecting flexibility and muscle adaptation [13]. Furthermore, the dry needling technique can restore ROM and muscle utilization patterns [18-20]. Accordingly, this study aims to investigate the effect of applying dry needling through the acupuncture technique (reduction technique) on hamstring muscle flexibility.

2. Materials and Methods

A total of 16 individuals with bilateral hamstring muscles shortness who were referred to Jame Clinic in Mehrshahr City, Iran, participated in the study and signed an informed consent. The sampling method was nonprobability convenience. The inclusion criteria were as follows: Women of 18 to 40 years old [21], having limitation in active knee extension test (≥20 degrees) [22], normal body mass index (18.5 to 29.9 kg/m²) [23], normal Q-angle (16-18 degrees) [24], negative navicular drop test [25], having no metabolic disorder (such as diabetes), having no coagulation problems, having no neurological problems (for example epilepsy), not being an athlete. Meanwhile, the exclusion criteria were as follows: Fear of needling, withdrawal from cooperation, failure to complete the treatment sessions, feeling too much pain or discomfort during the evaluation and treatment, and receiving secondary treatment during the treatment sessions.

The participants were randomly allocated to either the intervention (real dry needling) or a control (sham dry needling) group with a 1:1 allocation ratio. The randomization sequence was generated by the website [26]. Group assignment was concealed using sequentially numbered, opaque sealed envelopes. They were opened after the patient completed the baseline assessment. The study was a single-blinded, randomized controlled trial where participants were blinded to the treatment allocation, but not the therapist.

Given the outcome of active knee extension ROM in a pilot study, a priori power analysis (G*Power software, version 3.1.3) revealed a sample size of 14 participants to detect the within and between interaction effects. We assumed a medium effect size of 0.33 (Cohen f), obtained based on between-group variance=20.81 and within-group variance=189.062. We set the number of groups=2 (control and intervention), and the number of measurements=4 (pre, after the first, third, and fifth sessions of intervention). Also, we assumed the correlation among repeated measures=0.5 and non-sphericity correction ε =1. Moreover, sufficient power (80%) with α <0.05 was considered.

The outcome measure was right and left active knee extension ROM. The course of treatment was 5 sessions with 1-day intervals. The participants were assessed before and after the first, third, and fifth intervention sessions.

Study procedure

Measurement of knee joint range of motion

The participants laid on their backs and the opposite leg was fixed by a belt. The leg under evaluation was fixed by the fixator at a 90° angle and the fixator was fastened by the belt by attaching it to the bed (Figure 1). A standard 180-degree goniometer (Korean Saehan stainless steel goniometer) was used to measure ROM, such that the axis of the goniometer was placed on the lateral condyle of the femur, the fixed arm was placed along the greater trochanter, and the moving arm was placed along



Figure 1. Active knee extension test or hamstring muscles shortness test

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the lateral malleolus of the ankle. Then, the patient was asked to straighten the knee as much as possible. Next, the popliteal angle was measured. If the patient could not complete the last 20 degrees of knee extension, the test was positive [27, 28]. The popliteal angle was scored using an active knee extension test through a goniometer when the individual was trying to straighten the knee as much as possible [22].

Study groups

Real dry needling group

The course of treatment was 5 sessions with 1-day intervals. For needling, the patients lay in the prone position. A physiotherapist trained in dry needling applied needles to the motor points of the hamstring muscles (Figure 2). For the long head and short head of the biceps femoris, needles were applied into 2 points which were 30% and 60% of a straight line between the ischial tuberosity and the fibula head. In addition, for semitendinosus and semimembranosus, the needle was applied to a point that was 60% of the direct line between the ischial tuberosity and the medial condyle of the femur [13].

The dry needling used in the dry needling group was 50×30 mm in size, an Eco model made in China. For the reduction technique, needles were applied perpendicular to the motor points and rotated counterclockwise 360 degrees for 15 s to make one rotation per s. Then, the needles stay in the tissue for 20 min [17].



Figure 2. The locations of dry needling application

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Sham dry needling group

The course of treatment was 5 sessions with 1-day intervals. In this group, the tiny 13×20 mm needles were applied superficially at the location of the motor points without any manipulation and the needles stayed in the tissue for 20 min until the patient felt the needle and the treatment [29, 30].

Statistical analysis

The Shapiro-Wilk test was used to examine the normality of the data distribution. In this study, the data were normally distributed. Moreover, to ensure that the groups were comparable at baseline, an independent t-test was employed that was not statistically significant. A two-way ANOVA was conducted to analyze the main effect of time (before, after the first, third, and fifth sessions of intervention) and group (real and sham dry needling). If the main effect or interaction effect was significant, the post hoc pairwise comparisons were done with the Bonferroni corrected (corrected threshold of P=0.008). Moreover, the Cohen d was reported as a measure of the effect size (small=0.2, medium=0.5, large=0.8, and very large=1.2).

3. Results

Demographic results

The demographic variables had a normal distribution (P<0.05), hence, the independent t-test was used for the analysis (Table 1). The results showed that age (P=0.965), height (P=0.244), weight (P=0.874), and body mass index (P=0.251) were not significantly different between the two groups.

Right active knee extension range of motion

This variable was normally disturbed in the two study groups (P < 0.05). Also, the results of the independent t-

test showed that this variable was not significantly different between the two groups in the baseline (P<0.05), hence, the groups were comparable.

The main effect of time and group as well as the interaction effect of time and group are presented in Table 2. The analysis of variance showed, except for the group main effect, the time main effect and interaction effect were statistically significant (P<0.001).

The post hoc analysis reflected that in the real dry needling group, the right active knee extension ROM was significantly increased after the third session compared to before the intervention (P=0.002, d=2.40), after the fifth session compared to before the intervention (P<0.001, d=3.04), after the third session compared to after the first session (P=0.006, d=1.91), after the fifth session compared to after the fifth session compared to after the fifth session compared to after the fifth session (P=0.001, d=2.54), and after the fifth session compared to after the third session (P=0.001, d=2.64). However, in the sham dry-needling group, there was no significant difference across time (Table 3).

Left active knee extension range of motion

This variable was normally disturbed in the 2 study groups (P<0.05). Also, the results of the independent ttest showed that this variable was not significantly different between the two groups in the baseline (P<0.05); therefore, the groups were comparable.

The main effect of time and group as well as the interaction effect of time and group are presented in Table 2. The analysis of variance showed that, except for the group main effect, the time main effect and interaction effect were statistically significant (P<0.001).

The post hoc analysis reflected, in the real dry needling group, the left active knee extension ROM was significantly increased after the fifth session compared

Variables	Real Dry Needling			Sham Dry Needling				46	
	Mean±SD	Min	Max	Mean±SD	Min	Max	τ	df	Р
Age (y)	31.50±3.70	27	37	31.38±7.50	19	40	0.044	14	0.965
Height (cm)	165.00±8.91	155	178	159.88±7.90	145	168	1.217	14	0.244
Weight (kg)	63.25±8.84	53	77	64.00±9.69	50	82	-0.162	14	0.874
Body mass index (kg/m ²)	23.20±2.25	18.94	26.64	25.12±3.94	19.53	29.86	-1.198	14	0.251

Abbreviations: SD: Standard deviation; Min: Minimum; Max: Maximum; df: Degree of freedom.

Table 1. Demographic results

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Times	Mean±SD		Time Effect		Group Effect		Interaction Effect	
	Real DN	Sham DN	P	η²	Р	η²	Р	η²
Before	34.50±5.58	36.87±4.51	0.001*	0.792	0.111	0.171	0.001*	
After the 1 st session	33.62±6.02	36.80±4.50						0.608
After the 3 rd session	31.12±5.35	36.25±5.11						
After the 5 th session	29.12±4.96	35.75±4.97						
Before	31.50±5.9	34.00±4.95	0.001*	0.740	0.131	0.155	0.001*	
After the 1 st session	30.75±6.04	33.80±4.95						
After the 3 rd session	28.62±5.34	33.25±5.57						0.524
After the 5 th session	26.25±4.65	32.87±5.33						
	Before After the 1 st session After the 3 rd session After the 5 th session Before After the 1 st session After the 1 st session	TimesReal DNBefore34.50±5.58After the 1st session33.62±6.02After the 3rd session31.12±5.35After the 5th session29.12±4.96Before31.50±5.91After the 1st session30.75±6.04After the 3rd session28.62±5.34	TimesReal DNSham DNBefore34.50±5.5836.87±4.51After the 1 st session33.62±6.0236.80±4.50After the 3 rd session31.12±5.3536.25±5.11After the 5 th session29.12±4.9635.75±4.97Before31.50±5.934.00±4.95After the 1 st session30.75±6.0433.80±4.95After the 3 rd session28.62±5.3433.25±5.57	Times Time Real DN Sham DN P Before 34.50±5.58 36.87±4.51 P After the 1st session 33.62±6.02 36.80±4.50 0.001* After the 3rd session 31.12±5.35 36.25±5.11 0.001* After the 5th session 29.12±4.96 35.75±4.97 0.001* Before 31.50±5.9 34.00±4.95 0.001* After the 1st session 30.75±6.04 33.80±4.95 0.001* After the 3rd session 28.62±5.34 33.25±5.57 0.001*	Times Time Effect Real DN Sham DN P η^2 Before 34.50±5.58 36.87±4.51 $After the 1^{st} session$ 33.62±6.02 36.80±4.50 After the 1^{st} session 33.62±6.02 36.80±4.50 $After the 3^{rd} session$ 31.12±5.35 36.25±5.11 After the 3^{rd} session 29.12±4.96 35.75±4.97 0.001^{st} 0.792 Before 31.50±5.9 34.00±4.95 0.001^{st} 0.740 After the 1^{st} session 30.75±6.04 33.80±4.95 0.001^{st} 0.740 After the 3^{rd} session 28.62±5.34 33.25±5.57 0.001^{st} 0.740	Times Time Effect Group Real DN Sham DN P η^2 P Before 34.50±5.58 36.87±4.51 $After the 1^{st} session$ 33.62 ± 6.02 36.80 ± 4.50 $After the 1^{st} session$ 31.12 ± 5.35 36.25 ± 5.11 0.001^{st} 0.792 0.111 After the 3 rd session 29.12 ± 4.96 35.75 ± 4.97 0.001^{st} 0.792 0.111 After the 5 th session 29.12 ± 4.96 35.75 ± 4.97 0.001^{st} 0.792 0.111 After the 1 st session 30.75 ± 6.04 33.80 ± 4.95 0.001^{st} 0.740 0.131 After the 3 st session 28.62 ± 5.34 33.25 ± 5.57 0.740 0.131	TimesTime EffectGroup EffectReal DNSham DN P n^2 P n^2 Before34.50±5.5836.87±4.51 $After the 1st session33.62±6.0236.80±4.50After the 3rd session31.12±5.3536.25±5.110.001^40.7920.1110.171After the 3rd session29.12±4.9635.75±4.970.001^40.7920.1110.171Before31.50±5.934.00±4.950.001^40.7400.1310.155After the 1st session30.75±6.0433.80±4.950.001^40.7400.1310.155After the 3rd session28.62±5.3433.25±5.570.7400.1310.155$	Times Time Effect Group Effect Interaction Real DN Sham DN P η^2 P η^2 P Before 34.50 ± 5.58 36.87 ± 4.51 $P_{0.001}^{*}$ $P_{0.792}^{*}$ $P_{0.111}^{*}$ $P_{0.001}^{*}$ After the 1st session 31.12 ± 5.35 36.25 ± 5.11 0.001^{*} 0.792 0.111 0.171 0.001^{*} After the 3rd session 29.12 ± 4.96 35.75 ± 4.97 0.001^{*} 0.792 0.111 0.171 0.001^{*} Before 31.50 ± 5.9 34.00 ± 4.95 0.001^{*} 0.740 0.131 0.155 0.001^{*} After the 1st session 30.75 ± 6.04 33.80 ± 4.95 0.001^{*} 0.740 0.131 0.155 0.001^{*}

Table 2. The main and interaction effects for right and left knee

Abbreviations: ROM: Range of motion; SD: Standard deviation; DN: Dry needling.

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*There is a significant difference (P<0.008).

to before the intervention (P=0.002, d=2.27), after the fifth session compared to after the first session (P=0.005, d=1.99), and after the fifth session compared to after the third session (P=0.002, d=2.24). However, in the sham dry-needling group, there was no significant difference across time.

4. Discussion

This study aimed to investigate the effects of dry needling on muscle flexibility in individuals with hamstring muscle tightness. The results of this study showed that the application of dry needling using a reduction technique could improve muscle flexibility and increase the range of knee extension after 3 and 5 intervention sessions.

Studies have proved that applying static stretching techniques to shortened muscles also increases the crosssectional area of fibroblasts. In addition, this increase leads to a reduction in tension in shortened muscles. This occurs following the application of the dry needling technique in shortened tissues and similar responses occur in tissue fibroblasts. This mechanism may loosen the tissue following dry needling. When the dry needling is applied by twisting the needle into the tissue, it creates a mechanical stimulus by which the fascia network also affects the collagen and the underlying substance. This can be a factor in reducing muscle tension. For example, by examining the orientation of collagen, we have found that after needle application, the direction of tissue collagen is more paralleled and more direct compared to before the needle application [31].

Dry needling can also restore the pattern of muscle use to its original state [6]. This occurs by stimulating the motor points and increasing their excitability. In the muscular shortening, the reduction of the activity and excitability of motor points is probable. Dry needling can therefore improve the performance of motor points [18].

Studies have shown that manipulating connective tissue with dry needling can elicit cellular responses in connective tissue fibroblasts. When the needle is rotated, the collagen tissue around the needle forms a loop around the needle that stays in the tissue for 15 to 20 min. After the needle is removed, an internal tension is formed inside the connective tissue in response to this stable mechanical stimulus, causing the tissue to be released, and the fibroblasts expand their cytoskeletal tissue and regenerate it [33]. Thus, the viscoelastic properties of the desired soft tissue are restored [34].

Dry needling can also restore the ROM of joints that have been restricted following muscle shortening [6]. Dry needling increases ROM by creating relaxation while reducing tension in tissues that have become stiff [18].

Moreover, the mechanical signal generated by the mechanical stimulation of the needling causes changes in the cellular structure of actin, leading to the restoration of the normal cytoskeleton of actin. The changes that the needling causes in the tissue cells can be transmitted by

Table 3. Pair comparisons	within groups i	for right and	left knee
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		Groups				
Variables	Pair Comparisons	Rea	I DN	Sham DN		
		P	Cohen d	Р	Cohen d	
Right active knee extension ROM	Before-after the 1 st session	0.126	1.05	1.00	0.35	
	Before-after the 3 rd session	0.002*	2.40	0.570	0.68	
	Before-after the 5 th session	<0.001*	3.04	0.153	0.99	
	After the 1 st session-after the 3 rd session	0.006*	1.91	0.570	0.68	
	After the 1^{st} session-after the 5^{th} session	0.001*	2.54	0.153	0.99	
	After the 3^{rd} session-after the 5^{th} session	0.001*	2.64	0.621	0.66	
Left active knee extension ROM	Before-after the 1 st session	0.120	1.06	1.00	0.35	
	Before-after the 3 rd session	0.010	1.75	0.288	0.85	
	Before-after the 5 th session	0.002*	2.27	0.040	1.35	
	After the 1^{st} session-after the 3^{rd} session	0.027	1.46	0.288	0.85	
	After the 1^{st} session-after the 5^{th} session	0.005*	1.99	0.040	1.35	
	After the 3 rd session-after the 5 th session	0.002*	2.24	1.00	0.50	
There is a significant difference (P<0.008).				JMR	

ROM: Range of motion; DN: Dry needling.

the interstitial connective tissue to places farther away from the site of the needling. Accordingly, the needling widely affects the tissue, even at points farther from the site of needle entry [35]. With changes that the needle creates in the connective tissue surrounding neuro fibers can affect the function of these fibers and lead to longterm changes in their function with long-term changes in nerve connective tissue [36].

Few studies have examined the effect of the dry needling in the motor points of the hamstring muscles on the flexibility of this muscle in patients with hamstring muscle tightness and have reported an improvement in the ROM of the knee extension. Only three studies have used this technique. In two studies, they reported improved flexibility and increased ROM of knee extensions [13]. In one study, the needle was applied as needling in and out for 1 min, and flexibility was increased immediately after the intervention [13]. In another study, the needle was applied for 30 s and left for 5 min in the tissue. Accordingly, flexibility improved after 3 to 5 days [31]. In another study that used dry needling as needling in and out of the motor points for 1 min, no significant difference occurred in the ROM of the knee after 1 week of intervention [15].

The reason for not concluding the dry needling in the study conducted by Sadat et al. [15] is the use of the technique of rapid needling and the short time of staying the needle in the tissue. In the method of rapid needling, connective tissue gets less involved compared to when we use the needle rotation method. With the needle rotation technique, the skin, muscles, collagen, and connective tissue get involved and a complete mechanical stimulus occurs. The tissue that wraps around the rotated needle undergoes more tension compared to needling in and out; consequently, the tissue becomes loose after a strong tensile force. Due to the short duration of the needle remaining in the tissue, the loop and a common unit between needle and tissue do not form and mechanical stimulation is less and mechanical signal is not transmitted properly [35].

5. Conclusion

The results of this study showed that the application of dry needling on the motor points of the hamstring muscles can increase the flexibility of these muscles. This was a pilot study. Due to the therapeutic effects of the dry needling technique in improving muscle flexibility in people with hamstring muscle tightness, this intervention could be recommended in the clinical sets.

Study limitations

The limitations of this study were the time limitation of the study, a limited number of patients due to COVID-19 conditions, a limited population of patients under study, and the age limit of the patients. Meanwhile, the examiner was aware of the treatment process.

Suggestions for further research

It is suggested for further studies that the evaluators be blind to the intervention, the effect of more sessions of dry needling application be investigated, the duration of remaining effect of treatment in patients be evaluated, a follow-up study be conducted, and the intervention be examined for more patients.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Institutional Ethics Committee of the Tehran University of Medical Sciences (Code: IR.TUMS.MEDICINE.REC.1399.677)

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Authors' contributions

The authors contributed equally to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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