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Does Dry Needling Increase the Efficacy of Botulinum Toxin Injection in the Management of Post-Stroke Spasticity: A Randomized Controlled Study

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ABSTRACT

Introduction: The purpose of the current study was to investigate the antispastic efficacy of dry needling in combination with botulinum toxin-A injections.

Methods: Thirty stroke patients with elbow flexor spasticity were randomised into two groups; the patients treated with botulinum toxin-A injections and exercise into the BTX-A group, and patients treated with botulinum toxin-A injections, exercise, and dry needling in the BTX-A+Dry needling group. Spasticity was evaluated using the modified Ashworth scale and modified Tardieu scale before treatment, immediately after treatment, the third day after treatment, second week after treatment and at the third month after treatment. The upper extremity motor function was evaluated using the Fugl-Meyer upper extremity motor function scale.

Results: A statistically significant difference in all parameters was found after treatment in both groups compared to before treatment ($p<0.05$). In all evaluation parameters immediately after treatment, on the third day after treatment, the second week after treatment and the third month after treatment, a statistically significant difference in favour of the BTX-A+Dry needling group was achieved compared to before treatment ($p<0.05$)

Conclusion: Dry needling combined with botulinum toxin-A injections performed over a total of four sessions with three-day intervals, contribute to the antispastic effect. Also combined therapy is more effective and provides longer-lasting results.

Keywords: Stroke, hemiplegia, muscle spasticity, botulinum toxins, dry needling

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INTRODUCTION

A stroke is a clinical syndrome that can result in the loss of motor, sensory and cognitive functions or coma (1). Approximately 75% of stroke survivors develop a disability, with a primary cause being the presence of spasticity (2). Therefore, the effective treatment of spasticity accelerates functional recovery (3).

The botulinum toxin type A (BTX-A) injection is a safe and effective method for the treatment of focal spasticity (4). As its effects over the central and peripheral nervous system have been understood, dry needling has been recently introduced in clinical practice as a novel treatment of spasticity after a stroke (5–10). Studies on the efficacy of dry needling in the treatment of spasticity are limited in the literature and there is no data on its long-term efficacy in current studies (5–10). On the other hand, the effects of dry needling when combined with other antispastic treatment modalities have not yet been investigated. In the current study, the antispastic efficacy of dry needling with BTX-A injections were investigated.

Highlights

- **One of the main goals of post-stroke treatment is to control spasticity.**
- **The botulinum toxin type A injection is a safe and effective method.**
- **Dry needling contributes to the antispastic effect.**

METHODS

Study design

The study was designed as a prospective, randomised, single-blind study and conducted with the approval of the local ethics committee (Istanbul Haydarpasa Numune Training and Research Hospital Ethics Committee for Clinical Researches), dated October 9, 2017. The study protocol was compliant with the 1964 Declaration of Helsinki. Before the study, oral and written informed consent was obtained from all individual participants included in the study.

Participants

The 30 stroke patients included in the study were selected from 59 stroke patients admitted to the Physical Therapy and Rehabilitation Outpatient Clinic between October 11, 2017 and March 30, 2018.

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Inclusion criteria

1. Stroke patients between 20–80 years old
2. A modified Ashworth scale (MAS) spasticity level of two or three in the elbow flexor muscles of the hemiplegic side
3. First-time stroke patients
4. Patients without a joint pathology that would prevent passive movements of the involved elbow joint

Exclusion criteria

1. A mental health problem
2. The presence a of a cooperation-orientation limitation or neglect
3. The use of an oral antispastic agent
4. A BTX-A application in the last three months
5. A peripheral nerve injury in the affected side upper extremity
6. A wound in the area where the procedure will be applied
7. Patients with a contraindication for BTX-A application
8. Patients with a needle phobia

The patients were randomised into two groups using a random number table by a physician blinded to the study. Patients treated with BTX-A injections and exercise became the BTX-A group (n=15), and patients treated with BTX-A injections, exercise and dry needle application became the BTX-A+Dry needling group (n=15).

Interventions

Demographic data, medical history and physical examinations of the patients were recorded on the follow-up form. The BTX-A and the dry needle to be used were prepared by the same physician 10 minutes before the application in all patients. The vial containing 500 units (U) BTX-A (Dysport, Ipsen, France) was diluted with a sterile 2.5 ml saline solution containing 0.9% sodium chloride (11). In dry needling, two pieces of 0.30×40 mm sterile, single-use dry needles with a guide tube were used.

BTX-A group

Extremity positioning was conducted with the patient's shoulder on the hemiplegic side in mild abduction, the elbow in semi-flexion at submaximal tension and the forearm in supination. The ultrasound-guided BTX-A injection was performed with a 27-gauge, 0.40×50 mm sterile, disposable injection needle into the biceps brachii muscle using an in-plane approach (12, 13). All patients underwent 200 U BTX-A injections at three different points in the biceps brachii muscle. To determine these points on the biceps brachii muscle, the injection areas were previously defined with intense innervation areas taken into consideration (14). The SonoSite M-Turbo (FUJIFILM SonoSite, Inc. 21919 30th Dr. SE, Bothell, WA, 98021, USA) ultrasound system and 7–12 MHz linear probe were used in the applications.

BTX-A+Dry needling group

Patients who were included in this group underwent dry needling following the BTX-A injection. The antisepsis of the treatment area was restored without changing the patient's position. The point where the biceps brachii muscle was most tense and sensitive was primarily stabilised by superficial palpation, followed by pinch palpation. With the help of the guide tube, the skin was penetrated perpendicularly and the dry needle was inserted in a tight band in the spastic muscle. The penetration depth varied from patient to patient and was approximately 15–20 mm. Then, intramuscular dry needling was performed for 60 seconds with vertical 5–10 mm upward/downward movements, using the fast-in and fast-out cone shape technique, at a rate of about 1 hertz (1 upward-downward movement per second). This process was repeated for the two points in the biceps brachii muscle with the greatest tension (separately for its two heads). Dry needling was administered four times in total, with the first one administered immediately after the BTX-A injection and the remaining three in three-day intervals (the 3rd, 6th and 9th days after the BTX-A injection).

All injections and dry needling were performed by a physician with eight years of experience. All applications and evaluations were made in the morning between 9:00 a.m. and 12:00 a.m.

The exercise program was always accompanied by the same physiotherapist. The first session occurred immediately after the BTX-A injection (or after the first dry needling in the second group) and the second session occurred on the third day. Stretching exercises for the upper extremity spastic muscles and strengthening exercises for the antagonist muscles on the hemiplegic side were applied for 45 minutes per session. During the sessions, the patient's caregiver was present and the patient was informed that the exercise program should be continued regularly outside the hospital twice a day for 30 minutes. Patients were informed not to receive any additional treatment for spasticity until the study ended.

Assessment

Spasticity was evaluated using the MAS and modified Tardieu scale (MTS) before treatment (BT), immediately after treatment (IAT), on the third day after treatment (AT3), second week after treatment (AT2W) and at the third month after treatment (AT3M). Upper extremity motor function was evaluated using the Fugl-Meyer upper extremity motor function scale (FMMFS) BT, AT2W and AT3M. The assessor was blinded to the study.

Statistical Analysis

The statistical evaluation of the data obtained in this study was performed using the Microsoft SPSS version 22.0 statistical program. The Kolmogorov-Smirnov test was applied to determine whether the data were within normal distribution. Whether there was a statistically significant difference between the demographic data and baseline evaluations (MAS, MTS and FMMFS) was evaluated with the Chi-square test for discrete data and Mann-Whitney U test for continuous data.

The Friedman test was used to assess if there was a statistically significant difference between the intragroup data at the BT, IAT, AT3, AT2W and AT3M evaluations. A value of $p<0.05$ was considered significant. Post-hoc analysis was performed using the Wilcoxon test upon determination of a statistically significant change. The Bonferroni adjustment was applied and the significance coefficient was taken as $p<0.005$.

The statistical differences between the groups were evaluated by the Mann-Whitney U test during the BT, IAT, AT3, AT2W and AT3M evaluations. A correlational relationship between MAS and FMMFS values in all groups was tested using the Spearman test. The G*Power version 3.1.9.2 was used to determine the sample size. It was calculated with a 95% confidence interval, 0.05 alpha value and 95% power that 15 patients should be allocated to each group.

RESULTS

There was no statistically significant difference between the groups in terms of patient demographic data in this study (p>0.05) (Table 1).

No statistically significant difference was found between the two groups when pre-treatment parameters were evaluated (p>0.05).

A statistically significant difference was found in all parameters in all groups after treatment compared to before treatment ($p<0.05$). According to these results, in both groups the MAS, MTS quality of muscle reaction at V1 velocity (MTS V1X) and MTS quality of muscle reaction at V3 velocity (MTS V3X) scores and the MTS dynamic contracture angles decreased

Table 1. Inter-group comparison of demographic data and other data

Parameters	BTX-A Group (n=15)	BTX-A + Dry needling Group (n=15)	p
Age (years) (med and min/max)	59 (46/79)	64 (28/78)	>0.05
Duration of disease (months) (med and min/max)	37 (7/132)	23 (5/163)	>0.05
Sex (male/female)	11/4	9/6	>0.05
Etiology (ischemic/hemorrhagic)	12/3	11/4	>0.05
Hemiplegic side (right/left)	10/5	8/7	>0.05
Dominant hemisphere (right/left)	0/15	0/15	>0.05
Hypertension history (yes/no)	9/6	9/6	>0.05
Diabetes mellitus history (yes/no)	6/9	5/10	>0.05
Hyperlipidemia history (yes/no)	6/9	4/11	>0.05
Smoking history (yes/no)	3/12	3/12	>0.05

Coefficient of significance *p<0.05*; med, median; min/max, minimum/maximum

Table 2. Comparison of intra-group parameters BT, IAT, AT3, AT2W and AT3M Values in BTX-A group

Evaluation Parameter		BT (median) (min/max)	IAT (median) (min/max)	AT3 (median) (min/max)	AT2W (median) (min/max)	AT3M (median) (min/max)	p
Modified Ashworth Scale		2 (2/3)	2 (2/3)	2 (1+/3)	1 (1/2)	2 (1/3)	<0.05
Modified Tardieu Scale	**V1X**	2 (1/2)	2 (1/2)	2 (1/2)	1 (0/1)	2 (1/2)	<0.05
	V3X	3 (2/4)	3 (2/4)	3 (2/3)	2 (1/3)	3 (2/3)	<0.05
	V3Y	70 (30/84)	70 (32/87)	72 (36/89)	95 (72/120)	70 (40/90)	<0.05
	Dynamic Contracture	70 (56/110)	70 (53/108)	68 (51/104)	44 (20/68)	70 (50/100)	<0.05
FMMFS		17 (6/41)	NT	NT	24 (10/47)	21 (7/42)	<0.05

Coefficient of significance *p<0.05*; BT, before treatment; IAT, immediately after treatment; AT3, third day after treatment; AT2W, second week after treatment; AT3M, third month after treatment; V1X, modified Tardieu scale quality of muscle reaction at V1 velocity; V3X, modified Tardieu scale quality of muscle reaction at V3 velocity; V3Y, modified Tardieu scale muscle reaction angle at V3 velocity; FMMFS, Fugl Meyer motor function scale, NT, not tested.

Table 3. Comparison of intra-group parameters BT, IAT, AT3, AT2W and AT3M values in BTX-A + dry needling group

Evaluation Parameter		BT (median) (min/max)	IAT (median) (min/max)	AT3 (median) (min/max)	AT2W (median) (min/max)	AT3M (median) (min/max)	p
Modified Ashworth Scale		2 (2/3)	1 (1/2)	1 (1/2)	1 (0/2)	1+ (1/2)	<0.05
Modified Tardieu Scale	**V1X**	2 (1/2)	1 (0/2)	0 (0/2)	0 (0/2)	1 (0/2)	<0.05
	V3X	3 (3/4)	2 (1/3)	2 (1/3)	1 (0/3)	2 (1/3)	<0.05
	V3Y	70 (32/82)	100 (60/110)	105 (68/116)	110 (64/140)	88 (60/107)	<0.05
	Dynamic Contracture	70 (58/108)	40 (30/80)	35 (24/72)	28 (0/76)	50 (28/80)	<0.05
FMMFS		18 (6/33)	NT	NT	37 (20/48)	32 (17/43)	<0.05

Coefficient of significance *p<0.05*; BT, before treatment; IAT, immediately after treatment; AT3, third day after treatment; AT2W, second week after treatment; AT3M, third month after treatment; V1X, modified Tardieu scale quality of muscle reaction at V1 velocity; V3X, modified Tardieu scale quality of muscle reaction at V3 velocity; V3Y, modified Tardieu scale muscle reaction angle at V3 velocity; FMMFS, Fugl Meyer motor function scale.

Table 4. Inter-group comparison of BT, IAT, AT3, AT2W and AT3M values of evaluation parameters

Evaluation		BTX-A median (min/max)	BTX-A+Dry needling median (min/max)	p
IAT	MAS	2 (2/3)	1 (1/2)	<0.05
	MTS *V1X*	2 (1/2)	1 (0/2)	<0.05
	MTS *V3X*	3 (2/4)	2 (1/3)	<0.05
	MTS *V3Y*	70 (32/87)	100 (60/110)	<0.05
	Dynamic contracture	70 (53/108)	40 (30/80)	<0.05
AT3	MAS	2 (1+/3)	1 (1/2)	<0.05
	MTS *V1X*	2 (1/2)	0 (0/2)	<0.05
	MTS *V3X*	3 (2/3)	2 (1/3)	<0.05
	MTS *V3Y*	72 (36/89)	105 (68/116)	<0.05
	Dynamic contracture	68 (51/104)	35 (24/72)	<0.05
AT2W	MAS	1 (1/2)	1 (0/2)	<0.05
	MTS *V1X*	1 (0/1)	0 (0/2)	<0.05
	MTS *V3X*	2 (1/3)	1 (0/3)	<0.05
	MTS *V3Y*	95 (72/120)	110 (64/140)	<0.05
	Dynamic contracture	44 (20/68)	28 (0/76)	<0.05
	FMMFS	24 (10/47)	37 (20/48)	<0.05
AT3M	MAS	2 (1/3)	1+ (1/2)	<0.05
	MTS *V1X*	2 (1/2)	1 (0/2)	<0.05
	MTS *V3X*	3 (2/3)	2 (1/3)	<0.05
	MTS *V3Y*	70 (40/90)	88 (60/107)	<0.05
	Dynamic contracture	70 (50/100)	50 (28/80)	<0.05
	FMMFS	21 (7/42)	32 (17/43)	<0.05

Coefficient of significance *p<0.05*; min/max, minimum/maximum; BT, before treatment; IAT, immediately after treatment; AT3, third day after treatment; AT2W, second week after treatment; AT3M, third month after treatment; V1X, modified Tardieu scale quality of muscle reaction at V1 velocity; V3X, modified Tardieu scale quality of muscle reaction at V3 velocity; V3Y, modified Tardieu scale muscle reaction angle at V3 velocity; FMMFS, Fugl Meyer motor function scale.

significantly after treatment and the MTS muscle reaction angle at V3 velocity (MTS V3Y) and FMMFS scores increased significantly (Tables 2 and 3 near here).

In the BTX-A group, as a result of the intra-group dual comparison of the change in MAS, MTS V1X and MTS V3X parameters, only the decrease in AT2W values was found to be statistically significant compared to BT (p<0.005). In the BTX-A group, as a result of the intra-group dual comparison of changes in MTS V3Y and MTS dynamic contracture parameters, the changes in AT3 and AT2W values were found to be statistically significant compared to BT (p<0.005).

In the BTX-A+Dry needling group, as a result of the intra-group dual comparison of the changes in all the sub-parameters (V1X, V3X, V3Y and dynamic contracture) of the MAS and MTS, a statistically significant difference was found in all controls after treatment (IAT, AT3, AT2W and AT3M) compared to BT (p<0.005). At the same time in the BTX-A+Dry needling group, as a result of the intra-group dual comparison of the changes in the MTS V3Y and MTS dynamic contracture parameters, a statistically significant difference was found in AT3 and AT2W values compared to IAT (p<0.005).

In both the BTX-A and BTX-A+Dry needling groups, as a result of the intra-group dual comparison of the change in the FMFFS, a statistically significant difference was found in AT2W and AT3M values compared to BT (p<0.005).

At all evaluation parameters (MAS, MTS and FMMFS), the IAT, AT3, AT2W and AT3M controls showed a statistically significant difference in favour of the BTX-A+Dry needling group compared to BT (p<0.05) (Table 4 near here).

Relationships

There was a negative correlation between the decrease in MAS values at two weeks after treatment and three months after treatment and the increase in FMMFS values (p<0.05).

There was no patient who was unable to continue treatment for any reason. No complications were encountered during the study period.

DISCUSSION

In this study, which aimed to investigate the efficacy of dry needling applied with botulinum toxin injection and an exercise program on the spasticity and motor functions in the upper extremity of patients with post-stroke elbow flexor spasticity, the dry-needling applied with BTX-A injection and exercise seems to be a superior treatment method for reducing spasticity and improving upper extremity motor function both immediately after treatment and in the long term.

Studies on the effectiveness of dry needling for the treatment of spasticity in stroke patients are limited in the literature (5–10). We could not find any study evaluating the effectiveness of dry needling combined with BTX-A injections or with other antispastic treatments.

Herrero et al. applied dry needling to the right hand opponens pollicis, flexor carpi radialis, flexor digitorum superficialis, flexor digitorum profundus, biceps brachii and brachialis muscles of a four-year-old spastic tetraparetic patient who developed hypoxic ischemic encephalopathy due to perinatal fetal distress (15). They observed a clinically significant decrease in spasticity in all muscles after treatment with dry needling for nine sessions in the opponens pollicis muscle and for five sessions in the other muscles. Herrero et al. suggested that the significant reduction in resistance to passive movement might be due to a decrease in spasticity or changes in the viscoelastic properties of the muscle. It is possible that the antispastic effect obtained within minutes after dry needling may occur rapidly in the presence of neurohumoral mechanisms, including spinal reflex mechanisms or neurotransmitters functioning at the

nerve-muscle junction during the acute period (16). In the later days of treatment, possible structural changes in the spastic muscle tissue may be effective in maintaining the gained antispastic effect

Salom-Moreno et al. randomised 34 hemiplegic patients into two groups in their study (10). They applied dry needling for a single session to the affected-side gastrocnemius and tibialis anterior muscles of the 17 patients in the first group. They did not apply any treatment to the patients in the second group. The MAS was used to evaluate the ankle plantar flexor spasticity on the affected side. The measurements were performed prior to dry needling and 10 minutes after needling. They found a statistically significant decrease in ankle plantar flexor spasticity in the dry needling group after treatment. On the other hand, dry needling for only a single session and the follow-up parameters being evaluated only once, at ten minutes after treatment, left the question of the duration of significant improvement after treatment unanswered. In the current study, we applied a total of four sessions of dry needling over three-day intervals. We recorded the follow-up parameters both immediately after treatment and at certain intervals up to three months after treatment. Thus, we observed that the effectiveness of the treatment progressively increased with the repeated sessions and the antispastic effect lasted for at least three months.

Mendguita-Gomez et al. divided 20 patients who had developed shoulder spasticity and limitation of joint motion after a stroke into two groups (7). The first group was treated with a multimodal rehabilitation program combined with dry needling to the hemiplegic side pectoralis major, upper trapezius, infraspinatus and subscapularis muscles, and the second group was treated only with the multimodal rehabilitation program. In the group with dry needling applied for a total of three sessions at one-week intervals, the decrease in infraspinatus spasticity and the increase in joint range of motion in the abduction of the shoulder joint and external rotation were found to be statistically significant compared to the multimodal rehabilitation program-only group. In the current study, patients were also included in a rehabilitation program. In addition to the BTX-A and dry needling, we believe the rehabilitation program applied to patients also contributed to the results.

Calvo et al. aimed to show the effects of the dry needling for hypertonia and spasticity (DNHS) technique on the contractile elements of muscles in stroke patients (8). They applied dry needling to a 50-year-old patient who was spastic hemiplegic for 2.5 years. In the study, they applied dry needling to the spastic right side biceps brachii, triceps brachii, rectus femoris, semitendinosus, biceps femoris, medial gastrocnemius and lateral gastrocnemius muscles, using the fast-in and fast-out cone shape technique for a single session. Then they used tensiomyography to evaluate the contractile properties of the muscles. They found a decrease in muscle stiffness compared to before the treatment and emphasised that dry needling changed the contractile properties of the muscles towards a decrease in muscle stiffness and increased the muscles' maximum lengthening ability.

In the current study, the effect of dry needling on the contractile properties of the muscles were evaluated with MTS dynamic contracture angle. The MTS dynamic contracture angle (*V1Y – V3Y*) is the difference between the MTS *V1Y* and MTS *V3Y* angles; it is a measure of the velocity-dependent contraction response in the muscle. More specifically, the high dynamic contracture angle is defined when a passive movement is performed at low speed to the related joint of the muscle under examination and minimal resistance is seen. As the speed of the passive movement is increased, the resistance is increased. This demonstrates that this resistance is not directly caused by structural changes such as joint or muscle contracture but directly by the spasticity of the muscle itself. Therefore, although tensiomyography provides more objective data in the evaluation of *muscle stiffness*, the MTS dynamic contracture angle may be more valuable in the evaluation of *spasticity*. Tensiomyography is associated with the stiffness of muscles and tendons, not directly associated with spasticity (17).

Fakhari et al., in a single group pretest-posttest study of 29 stroke patients with wrist flexor spasticity, applied dry needling using the fast-in and fast-out cone shape technique to the flexor carpi ulnaris and flexor carpi radialis muscles for one session each with a 60-second duration (5). In the evaluation, MAS, Hmax/Mmax ratio, H reflex latency, passive resistance force, wrist passive and active range of motion and the box and block test were used. The results were recorded before treatment (T0), immediately after treatment (T1) and at the first hour after treatment (T2). The number of successfully transferred blocks in the box and block test was significantly increased after the treatment and continued for the first hour after the treatment. In addition, a statistically significant decrease in Hmax/Mmax ratio and a significant increase in H reflex latency was detected. There was a statistically significant negative correlation between MAS scores and the box and block test. As a result, in the Fakhari et al. study of patients with chronic stroke, a single session of dry needling reduced the spasticity in the wrist flexors, suppressed alpha motor neuron excitability and improved upper extremity motor function. The effects of dry needling continued for the first hour after the application. In accordance with the results found in the study by Fakhari et al., we observed that the elbow flexor spasticity decreased immediately after treatment in the patients treated with dry needling and BTX-A. Similarly, there was a negative correlation between the MAS and FMMFS values (Spearman test, $p<0.05$). We found an increase in upper extremity motor function with decreased spasticity. This is an indicator of the negative effect of spasticity on motor function.

Chin et al. controlled the accuracy of the BTX-A injection applied by a manual palpation technique with an electric muscle stimulator (EMS) in 226 children with cerebral palsy (18). They detected the accuracy of manual palpation technique >75% for gastro-soleus, 67% for hip adductors, 46% for medial hamstring, 11% for tibialis posterior, 62% for biceps brachii, 35% for pronator teres, 22% for adductor pollicis, 16% for flexor carpi ulnaris and 13% for flexor carpi radialis, and emphasised only the values for the gastro-soleus complex were acceptable. Picelli et al. found that EMS and ultrasonography (USG) were superior to manual palpation in toxin injections applied to the forearm muscles of patients with chronic stroke, but they found no significant difference between EMS and USG (19). Ata and Dincer applied toxin injections in patients who developed upper extremity spasticity after a stroke and used USG and EMS simultaneously (20). They found a significant decrease in spasticity in all patients and a significant increase in upper extremity motor function and functionality compared to the beginning. In this study, all BTX-A injections were performed using the ultrasound-guided in-plane method. In both groups, there was a statistically significant increase in upper extremity motor function and a decrease in spasticity after treatment with no application-induced complications during the treatment.

One of the main goals of post-stroke treatment is to control spasticity in order to make the patient as independent as possible by improving motor function and minimizing complications. In this study, we suggest that when combined with the current treatment modalities, dry needling in the treatment of post-stroke spasticity can be an effective and reliable complementary treatment option with low cost and very low side effect potential.

It is possible that the dry needling may have a placebo effect (16). Therefore, the lack of a sham dry needling group can be considered a limitation of this study. However, considering the remote and distal effect of dry needling, it does not seem possible to determine a sham technique.

Needling applied to any part of the body may also show activity in the area where the main treatment is given (21).

As a result, the data obtained in the study was compatible with the results that BTX-A injection to the upper extremity together with a rehabilitation program decreased post-stroke spasticity, improved upper extremity motor function, and this improvement continued for three months after injection. In addition, dry needling combined with BTX-A injection and performed for a total of four sessions at three-day intervals, contributes to the antispastic effect as a fast-acting, effective, long-term treatment.

This research was presented as an oral presentation at the 6th Medical Rehabilitation Congress (November 8-11, 2018, Ankara, Turkey).

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki.

Informed Consent: Before the study, oral and written informed consent was obtained from all individual participants included in the study.

Peer-review: Externally peer-reviewed

Author Contributions: Concept – EA, MK; Design – MK, EA, FY; Supervision – FY, MK; Resources – MK, EA; Materials – FY, MK; Data Collection and/or Processing – MK, EA; Analysis and/or Interpretation –MK, EA, FY; Literature Search – MK, EA; Writing Manuscript – MK, EA, FY; Critical Review – EA, FY.

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