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Changes in stiffness at active myofascial trigger points of the upper trapezius after dry needling in patients with chronic neck pain: a randomized controlled trial

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Abstract

Background/objective: Since, to our knowledge, the effects of dry needling (DN) on active myofascial trigger point (MTrP) stiffness have not been analyzed previously with shear wave elastography (SWE), our aim was to compare the effects of a single session of DN and sham DN applied to the most active MTrP located in the upper trapezius muscle on clinical outcomes.

Methods: A randomized, double-blinded sham-controlled trial was conducted; 60 patients were randomized into an experimental (DN) or sham (sham DN) group. Baseline data including sociodemographic and clinical characteristics were collected. SWE and pain pressure thresholds (PPTs) at the MTrP and a control point located 3 cm laterally were the main outcomes assessed before and 10 min after the interventions.

Results: Patients receiving DN interventions experienced greater increases in the control point PPTs immediately after receiving the intervention compared with sham DN ($p < 0.05$), but no differences were found for the MTrP ($p > 0.05$). Post-intervention PPT improvements were found at both locations for both groups ($p < 0.01$). No significant changes for either MTrP or control locations were found for SWE outcomes in either group (all $ps > 0.05$). No significant within-group SWE differences were found in the DN or sham DN groups ($p > 0.05$).

Conclusion: A single session of DN or sham DN applied to active MTrPs located in the upper trapezius muscle produced no detectable changes in stiffness at the MTrP or control locations. Real DN induced an immediate analgesic response at both MTrP and control locations, while sham DN induced an immediate MTrP response.

Trial registration number: NCT04832074 (ClinicalTrials.gov).

Keywords

dry needling, elasticity imaging techniques, myofascial pain syndrome, myofascial trigger point, neck pain, pain pressure threshold, shear wave elastography

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Introduction

Shear wave elastography (SWE) is an ultrasound-based imaging technology sensitive to tissue stiffness, which has been developed in recent years to enable a quantitative assessment.[1] A shear wave is a transverse wave that occurs in an elastic medium when it is subjected to a shear force (defined as the change in the shape of a substance layer without volume change, produced by a pair of equal forces working in opposite directions along the two opposed sides of the layer). After the shear interaction, the initial layer will resume its original shape, while the adjacent layers undergo shear, which causes further shifting and propagation as a transverse shear wave.[2] Therefore, the production of this radiating force by the probe rather than the operator makes this method more operator-independent and reproducible. In addition, SWE allows measurement of several parameters (e.g. Young's modulus (kPa) and local shear wave speed (m/s)).[3]

Currently, SWE is widely used to assess several structures, including the liver, breast, thyroid, kidney, prostate and lymph nodes.[4] Although the clinical applications of SWE for assessing the musculoskeletal system are limited, studies assessing tendons and muscles have been increasing in the last few years.[5,6] In fact, recent studies have assessed myofascial trigger points (MTrPs) using SWE.[7–10]

An MTrP is defined as "a hyperirritable spot in skeletal muscle that is associated with a hypersensitive palpable nodule in a taut band which is painful on manual compression and can give rise to characteristic referred pain, referred tenderness, motor dysfunction and autonomic phenomena."[11] Although the etiology of neck pain seems to be multifactorial, patients with chronic neck pain exhibit increased presence and tenderness of MTrPs in the upper quarter muscles (e.g. upper trapezius, infraspinatus and levator scapulae).[12] In fact, there is evidence to suggest that the prevalence and pain sensitivity of MTrPs are sensitive enough to make distinctions between patients with chronic non-traumatic neck pain and healthy controls.[13]

Since neck pain is the fourth highest ranking condition in terms of number of years lived with disability (with an estimated point prevalence of 20%, lifetime prevalence up to 70% and high recurrence rates), dry needling (DN) of MTrPs has been widely applied and analyzed in both the research and clinical fields to reduce pain and disability in patients with chronic neck pain.[14] Although the level of evidence is weak for effects on functionality and quality of life, recent systematic reviews suggest a role of DN in the treatment of neck pain patients with trigger points in the upper trapezius muscle in the short and medium term.[14–16]

After an extensive literature search, we deemed the available evidence regarding the effects on stiffness of DN targeting active MTrPs in patients with unilateral chronic neck pain to be lacking and to have methodological flaws.[9,10] Therefore, our aim was to compare the effects of a single session of real DN versus sham DN on pain pressure threshold (PPT) and SWE outcomes (Young's modulus and local shear wave speed) at the MTrP and a control location in patients with unilateral chronic neck pain.

Methods

Study design

We conducted a randomized and double-blinded clinical trial comparing the effects of a single session of real DN (approaching the most active MTrP located in the upper trapezius muscle) versus sham DN in patients with unilateral chronic neck pain. The primary outcomes of the study were the immediate changes in local shear wave speed and Young's modulus (assessed by SWE) and PPTs (assessed with algometry). This clinical trial followed the CONsolidated Standards Of Reporting Trials (CONSORT) guidelines for pragmatic clinical trials[17] and the Enhancing the QUAlity and Transparency Of health Research (EQUATOR) guidelines.[18] This study was conducted according to the Declaration of Helsinki, approved by the Institutional Ethics Committee of Clinical Research of Francisco de Vitoria University (identification No. 38/2021) and prospectively registered at ClinicalTrials.gov on 5 April 2021 (registration No. NCT04832074).

Participants

A consecutive sample of patients with chronic unilateral neck pain was screened for eligibility criteria between 28 April 2021 and 3 May 2021 from a private clinic of Francisco de Vitoria University (Spain) following advertisement via local flyer announcements. To be eligible for participation, volunteers had to be between 18 and 65 years old, have been experiencing unilateral neck pain for at least 3 months, have a Neck Disability Index (NDI) score >8, have a Visual Analogue Scale (VAS) score >3, and have at least one active MTrP located in the upper trapezius muscle. Exclusion criteria included whiplash injury, previous cervical surgery, cervical radiculopathy or myelopathy, diagnosis of fibromyalgia, analgesic treatment (e.g. physiotherapy or drugs) during the week prior to their participation, psychiatric disorders, or any contraindication to DN (e.g. fear of needles or anticoagulants). Data collection started on 3 May 2021 and was completed on 18 May 2021. Participants were not involved in the design, conduct, reporting or dissemination plans of our research.

Randomization and masking

Participants were randomly assigned to experimental (real DN) or sham (sham DN) groups using a random-number generator (Research Randomizer, version 4.0). Individual and sequentially numbered cards with the random assignment

were folded in sealed opaque envelopes for allocation concealment. The envelope was selected by one external researcher who proceeded with appropriate allocation, which was only revealed to the therapist conducting the sham or real DN after baseline data collection. The rater and participants were blinded to group allocation.

Procedures

MTrP identification. A single clinician with 10+ years of experience in myofascial pain syndrome management used physical examination to confirm active MTrPs using a palpation protocol that has been shown to reliably identify MTrP locations in the upper trapezius muscle.[19] This protocol consisted of manual palpation and responses of the patients to specific questions about painful symptoms (i.e. if the manual compression reproduced the patient's symptoms and if there was any referred pain).[11] We followed the criteria for identification of an active MTrP provided by Fernández-de-las-Peñas and Dommerholt,[11] in which an active MTrP is defined as one that either partially or completely reproduces any symptom experienced by the patient upon stimulation.

DN intervention. The active MTrP located in the upper trapezius muscle previously identified was needled by the same clinician with 10+ years of experience. If more than one active MTrP was identified, we selected the most symptomatic one (considered to be the one producing the most familiar pain, as described by the patient). Patients received DN using 0.25 mm × 25 mm disposable stainless steel solid filiform needles (Agupunct APS®, Barcelona, Spain) applying the technique described by Gerber et al.[20]

The participants were placed in the prone position. After cleaning the skin with 2% chlorhexidine (Lainco®, Barcelona, Spain), the plastic guide tube was placed over the MTrP with the dominant hand while the taut band was localized between the thumb and index finger of the non-dominant hand. Tapping with the dominant index finger was performed to insert the needle. Afterwards, the needle was moved to the muscle around the bundle and repeatedly moved in and out until a single twitch response was induced. If no twitch response was induced, needling was stopped after two or three in–out fast movements.[20,21]

Sham DN intervention. For the sham DN intervention, a similar approach was used by the same experienced examiner, but the skin was not pierced. A telescopic Park's sham device was used to apply sham needling (Dongbang Medical Co., Ltd., South Korea). The guide tube was pressed against the skin mark and the sham needle was allowed to drop. The handle was tapped briskly, but the (blunted) needle tip did not break the skin. The sham needle retracted within the guide tube and was pressed against the skin, simulating the quick "in and out" technique applied in the real DN group. The patients were unable to view the actual needling procedure during treatment because of their prone positioning on the table.

Outcomes

Baseline. At baseline, a standardized history with sociodemographic data (e.g. sex, age, height, weight and body mass index) was collected. In addition, prior to the subjects' participation in the study, subjective neck pain perception and neck disability were assessed.

To subjectively assess neck pain perception, patients were asked to identify their level of pain on a 100-mm VAS (where 0 means absence of pain and 100 the worst imaginable pain) by calculating the mean of three measurements (worst perceived pain during the last week, lowest pain intensity during the last week, and current pain).[22] Scores <3.4 were considered to represent mild pain, 3.5–6.4 moderate pain and >6.5 severe pain, respectively.[22]

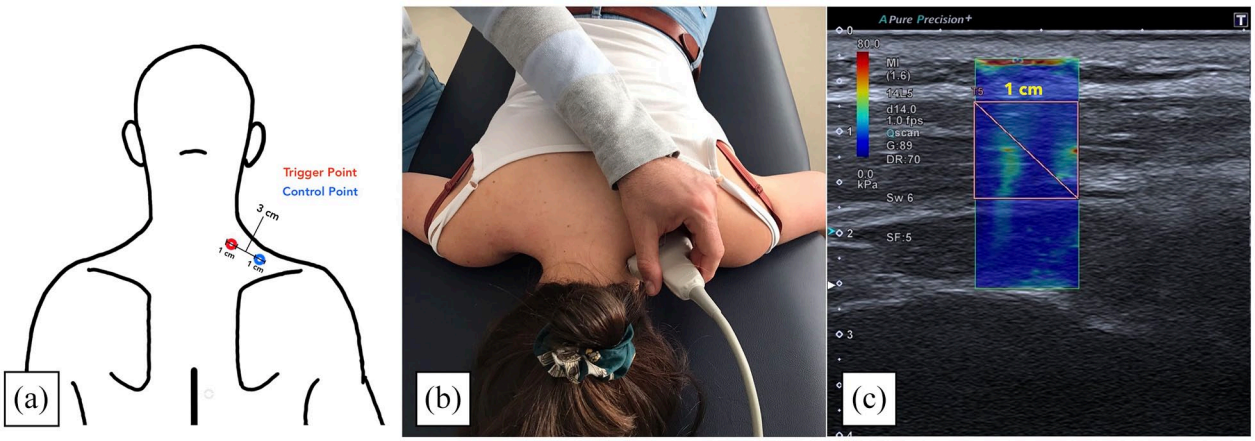
To assess neck disability, the NDI was used, since it is a valid tool for measuring perceived disability associated with neck pain.[23] Scores of 0 to 8 were considered to represent no disability, 10 to 28 mild disability, 30 to 48 moderate disability, 50 to 68 severe disability and 70 to 100 complete disability, respectively.[24]

Primary outcomes. Outcomes were evaluated before and immediately after the intervention by a single assessor blinded to subjects' group allocation. The primary outcomes measured were the local shear wave speed and Young's modulus, as assessed by SWE (both calculated automatically from the Ultrasound Imaging device after selecting the region of interest (ROI)), and PPT (as assessed by algometry).

All SWE assessments were performed by a sonographer with 10+ years of experience in musculoskeletal evaluation using a Canon Aplio A with a PLT-1005-BT 14L5 (5–14 MHz) transducer (Canon Medical Corp., Japan). All participants were positioned in the same orientation used for the MTrP identification protocol. All images were acquired with the transducer oriented longitudinally to the muscle fibers, since this procedure has shown good to excellent intra-session repeatability (intraclass correlation coefficient (ICC) >0.80) and moderate inter-rater and inter-session reproducibility (ICC $=0.66$–0.74).[25] Two images were obtained from each patient, one focused on the MTrP area and one focused on a control point located 3 cm lateral to the MTrP (Figure 1(a)). Therefore, the probe was centered over each of both upper trapezius measurement points in turn (Figure 1(b)).

In order to avoid bias regarding the MTrP location based on the stiffness scale, ROIs for shear wave data were selected, covering: (1) the superficial and deep internal echogenic fascia of the upper trapezius; and (2) 1 cm of width, corresponding to the algometer surface (Figure 1(c)). The sonographer was blinded to both group allocation (experimental vs sham)

Figure 1. Shear wave elastography (SWE) measurements: (a) myofascial trigger point (MTrP) and control point measurement locations, (b) transducer positioning (imaging acquisition of the control point located 3 cm lateral to the active MTrP), and (c) SWE image: (region of interest) assessment.



and the point assessed (MTrP vs control) as they were not in the room during the interventions, and an identical pen was used by the clinician to mark MTrPs and control points.

Upper trapezius PPTs were analyzed using a digital Wagner FDX algometer (with 1 cm^2 surface area) in both MTrP and control locations. Algometry assessment of MTrPs is a reliable method to assess changes in pain sensitivity at this location.[26] All the PPT assessments were performed by the same assessor and calculated as the mean of three trials. The applied pressure was increased at a rate of 1 kg/s. Standardized instructions given to each subject were as follows: "I am going to push on your body at 2 places. If you feel pain, not pressure, say 'now' and I will stop."[27]

Secondary outcomes. There were no secondary outcomes for this study. VAS and NDI scores were not measured beyond baseline.

Treatment side effects

Participants were asked to report any adverse events or sequelae (defined as short–medium term symptoms perceived as unacceptable *by* the patient or that required further treatment) experienced during or after the interventions (over the 1-month duration of this study).[28]

Sample size determination

We performed an a priori sample size estimation using G*Power (v.3.1.6) software and a two-tailed test. We determined that 54 participants would be required to detect an effect size of $d = 0.95$ for PPT (DN = 2.39 ± 0.81, sham DN = 1.49 ± 0.96, based on a similar study)[29] at a significance level of 5% ($\alpha = 0.05$) at 95% power. The sample size is similar to previous calculations provided by Sánchez-Infante et al.[9] and Gerber et al.,[20] who considered a minimum sample size of 52 participants to be appropriate.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 25 (IBM Corporation, Armonk, NY, USA), with a significance level of $p < 0.05$. After verifying data normality, descriptive statistics were used. Normally distributed data were described by mean, standard deviation (SD) and 95% confidence interval (CI). Between-group comparability at baseline (for both gender and intervention groups) was assessed with the independent *t*-test for continuous data. Analysis of covariance (ANCOVA) using baseline values as covariates has been shown to be more powerful than repeated-measures analysis of variance (RM ANOVA) when random group assignment is used.[30] Therefore, a 2 × 2 ANCOVA with time (before or after treatment) as the within-subjects factor, group (DN or sham DN) as the between-subjects factor, and baseline values as covariates, was used to examine the effects of the interventions. Given our multiple primary outcomes, a Bonferroni post hoc correction for multiple testing was carried out when analyzing specific differences between and within groups, such that only $p < 0.017$ (= 0.05/3) was assumed to be significant. The effect size was calculated as the partial eta squared (η_p^2) if post hoc analyses were significant. A $\eta_p^2 = 0.01$ was considered small, 0.06 medium, and 0.14 large, respectively.[31]

Results

Sixty-six participants were initially recruited in April 2021. Six participants were excluded for the following reasons: fear of needles ($n=3$), use of pharmacological treatment ($n=2$) and refusal to participate for personal reasons ($n=1$). Sixty patients with unilateral chronic neck pain were finally included and randomized into one of two groups: DN ($n=32$) or sham DN ($n=28$). None of the participants were lost or excluded during the study (Figure 2) or reported adverse effects during the study. Both groups were comparable at baseline (Table 1).

Prior to the intervention, significant PPT differences between both measurement points (MTrP and control) were found in both groups (DN, $p<0.001$; sham DN, $p<0.01$). However, no differences between points were found immediately after the intervention for either group ($p>0.05$). The mixed-model ANCOVA revealed a significant group × time interaction for the control point PPTs ($F=6.718$, $p<0.01$, $\eta\pi^2=0.102$); patients receiving the DN intervention experienced greater increases in the control point PPT immediately after receiving the intervention compared with sham DN (Table 2). However, no significant group × time interaction for the MTrP-PPTs was found. Post hoc analyses revealed significant within-session PPT increases in the DN group (MTrP, $p<0.001$; control point, $p<0.01$) and significant within-group changes in the sham DN group (MTrP, $p<0.01$).

Data regarding the SWE outcomes are reported in Table 3. At baseline, neither Young's modulus nor shear wave speed differed between the MTrP and control point

Figure 2. Participant flowchart (CONSORT).

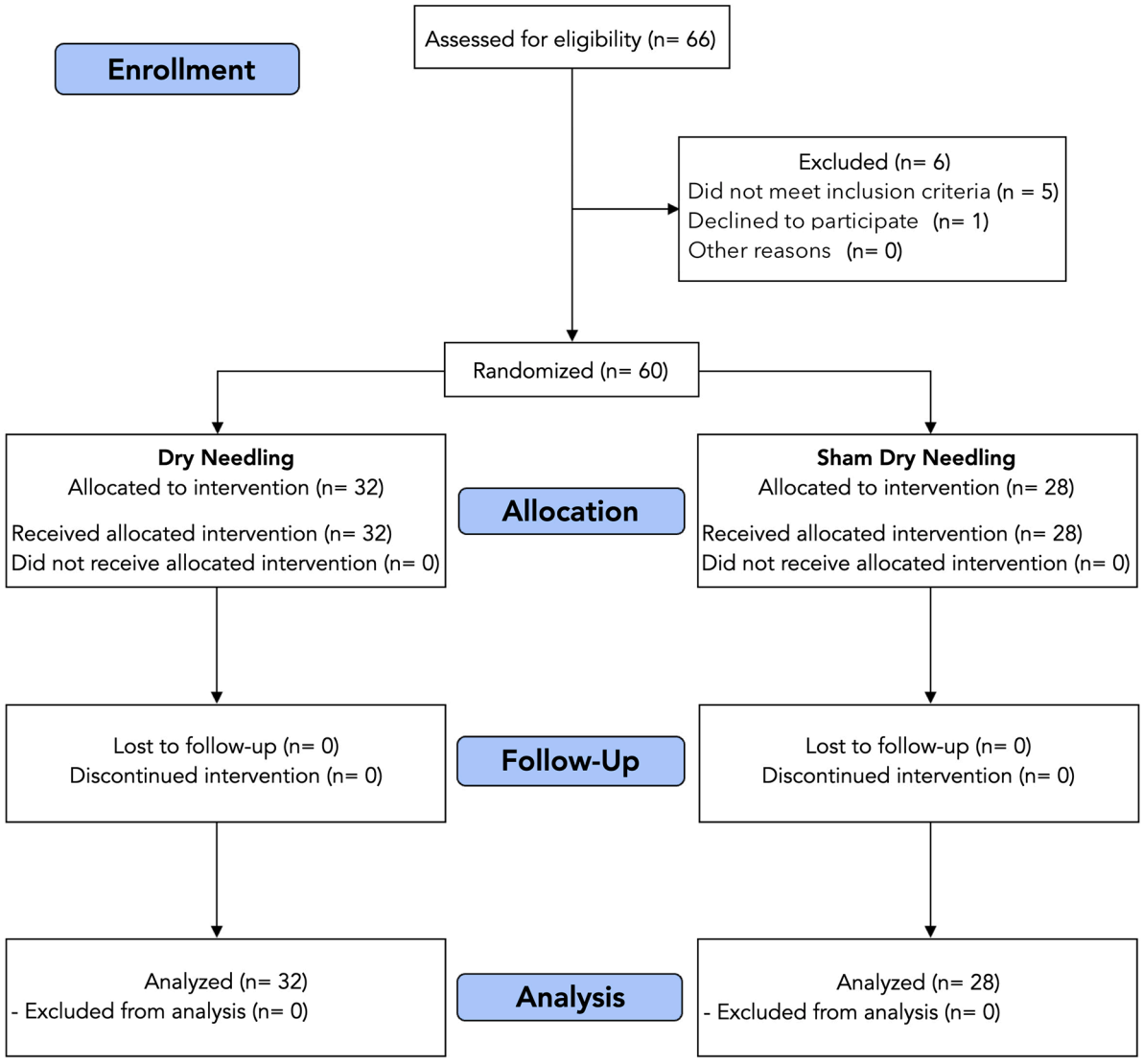


Table 1. Participant characteristics at baseline.

	Subjects (*n*)	Age (years)	Height (m)	Weight (kg)	BMI (kg/m^2)	NDI (%)	VAS (0–10)
Sample	60	21.9 ± 5.9	1.70 ± 0.10	64.2 ± 12.5	21.9 ± 2.6	17.5 ± 7.5	4.2 ± 1.1
Gender							
Male	16	23.5 ± 2.2	1.82 ± 0.07*	79.0 ± 9.4*	23.6 ± 2.4*	18.1 ± 6.8	4.0 ± 1.0
Female	44	21.3 ± 6.8	1.65 ± 0.07	58.8 ± 8.5	21.3 ± 2.5	17.2 ± 7.7	4.4 ± 1.1
Intervention groups							
Dry needling	32	22.3 ± 7.9	1.69 ± 0.11	61.9 ± 13.3	21.4 ± 2.6	17.9 ± 7.5	4.4 ± 1.0
Sham dry needling	28	21.4 ± 2.3	1.71 ± 0.10	66.8 ± 11.1	22.5 ± 2.6	17.0 ± 7.4	4.1 ± 1.2

BMI: body mass index; NDI: Neck Disability Index; VAS: Visual Analogue Scale.
Values are mean ± SD.
*Significant differences between groups ($p < 0.001$).

Table 2. Intervention effects: upper trapezius PPTs.

Variable		Timing of measurement	Dry needling	Sham dry needling	Within-group differences	MTrP-control point differences	Between-group differences
PPT (kg/cm^2)	MTrP	Pre-intervention	1.82 ± 0.77	1.84 ± 0.95	0.53 [0.00, 1.05][a],*	Pre-intervention: 0.53 [0.01, 1.05][c],*	0.14 [–0.49, 0.20]
		Post-intervention	2.36 ± 1.26	2.23 ± 1.30	0.38 [–0.21, 0.99][b],**	0.96 [0.28, 1.63][d],**	$F = 0.739$, $\eta_p^2 = 0.012$
	Control point	Pre-intervention	2.36 ± 1.10	2.80 ± 1.15	0.41 [0.17, 0.66][a],**	Post-intervention: 0.41 [–0.29, 1.12][c]	0.51 [0.11, 0.91]**
		Post-intervention	2.78 ± 1.24	2.70 ± 1.55	–0.10 [–0.43, 0.23][b]	0.47 [–0.23, 1.17][d]	$F = 6.718$, $\eta_p^2 = 0.102$

PPT: pain pressure threshold; MTrP: myofascial trigger point; SD: standard deviation; CI: confidence interval.
Data are expressed as mean ± SD [95% CI].
[a]Dry needling: within-group difference.
[b]Sham dry needling: within-group difference.
[c]MTrP-control point differences for dry needling group.
[d]MTrP-control point differences for sham dry needling group.
*$p < 0.001$; **$p < 0.01$.

locations in either group (all $p > 0.05$). The mixed-model ANCOVA showed no significant group × time interaction for MTrP or control point location SWE outcomes in either group (all $p > 0.05$). In addition, post hoc analyses revealed no significant within-session SWE changes in either group (MTrP and control point, $p > 0.05$).

Discussion

The present study assessed the effects of real and sham DN on pain and SWE outcomes in patients with unilateral chronic neck pain and found that a single session of DN or sham DN induced no changes in any of the SWE outcomes assessed. In addition, although both interventions increased MTrP-PPTs, only the real DN group showed increased PPTs at the control point location.

The positive effects of DN for the management of chronic neck pain are documented in the current literature.[14–16] Although the analgesic effects of DN in the short term are well known, evidence regarding effects on muscle stiffness is scarce. One previous study showed that a single DN intervention induced a Young's modulus reduction response at the MTrP location.[10] However, the sample size of this study was limited, there was no control point comparison within the upper trapezius muscle, and no comparative groups were assessed. In addition, one recent clinical trial found that a DN intervention at latent MTrPs located in the upper trapezius induced a greater reduction in muscle stiffness and increase in PPT than sham DN.[9]

Surprisingly, although there were effects on pain outcomes, our results showed no effect of DN or sham DN on SWE outcomes. Since MTrPs are defined as "hard palpable nodules within a taut band" and characterized by "local or referred tenderness associated with pain,"[11] it would be logical to find stiffness and PPT differences between an MTrP and a control point within the same muscle. However, even if MTrPs showed increased pain sensitivity (lower PPT) compared with control point locations, we did not find differences in stiffness. Our results are consistent with a previous study, which reported that

Table 3. Intervention effects on shear wave elastography: Young's modulus and shear wave speed.

Variable		Timing of measurement	Dry needling	Sham dry needling	Within-group differences	MTrP-control point differences	Between-group differences
Young's modulus (kPa)	MTrP	Pre-intervention	14.03 ± 3.66	14.39 ± 4.78	0.27 [–1.04, 1.59][a] 0.70 [–1.06., 2.47][b]	Pre-intervention: 0.02 [–1.97, 2.02][c] 2.35 [0.49, 5.19][d] Post-intervention: 0.51 [–1.47, 2.49][c] 0.84 [–2.44, 4.13][d]	0.43 [–1.71, 2.58] $F = 0.162$; $\eta^2_p = 0.003$
		Post-intervention	14.31 ± 4.10	15.10 ± 6.44			
	Control point	Pre-intervention	14.00 ± 4.18	16.75 ± 5.95	0.20 [–1.65, 1.25][a] 0.80 [–0.65, 2.27][b]		0.60 [–1.41, 2.62] $F = 0.358$; $\eta^2_p = 0.006$
		Post-intervention	13.80 ± 3.57	15.94 ± 6.05			
Shear wave speed (m/s)	MTrP	Pre-intervention	2.09 ± 0.29	2.05 ± 0.36	0.02 [–0.08, 0.13][a] 0.05 [–0.06, 0.17][b]	Pre-intervention: 0.01 [–0.13, 0.16][c] 0.19 [–0.01, 0.39][d] Post-intervention: 0.05 [–0.09, 0.19][c] 0.07 [–0.13, 0.28][d]	0.03 [–0.13, 0.19] $F = 0.148$; $\eta^2_p = 0.003$
		Post-intervention	2.12 ± 0.30	2.11 ± 0.41			
	Control point	Pre-intervention	2.07 ± 0.30	2.24 ± 0.40	0.00 [–0.10, 0.11][a] 0.06 [–0.04, 0.16][b]		0.05 [–0.09, 0.20] $F = 0.476$; $\eta^2_p = 0.008$
		Post-intervention	2.06 ± 0.25	2.18 ± 0.37			

MTrP: myofascial trigger point; CI: confidence interval.
Data are expressed as mean ± SD [95% CI].
[a]Dry needling: within-group difference
[b]Sham dry needling: within-group difference
[c]MTrP-control point differences for dry needling group
[d]MTrP-control point differences for sham dry needling group

hardness of the trapezius muscle does not directly reflect subjective shoulder stiffness.[32]

Despite the fact that palpation is widely used to assess differences in stiffness, palpation is a highly subjective test which could introduce bias and, therefore, objective methods (with high sensitivity, specificity, reliability and validity) are needed.[33] SWE is a feasible tool to assess within-session changes in the upper trapezius muscle stiffness under resting conditions,[25] and has been shown to be a clinically relevant outcome for the assessment of patients with neck pain based on a comparison between a sample of patients with bilateral chronic neck pain (with active MTrPs) and pain-free controls (with latent MTrPs).[34] Although this study found no correlation between SWE and myofascial pain syndrome severity, general muscle stiffness has been demonstrated to be more discriminative in patients versus controls than stiffness assessments at specific MTrP locations.[34]

However, SWE has been shown to be only moderately reliable when performed under rigorous conditions (e.g. assessment in a longitudinal plane and reporting shear wave speed).[35] Therefore, studies assessing transverse planes[9,10] should be interpreted carefully. Since no SWE effects were induced by the single session interventions in this study, further research assessing the clinical relevance of SWE (e.g. subjective pain perception, referred pain area, neck disability and PPTs) comparing clinical and healthy populations is needed.

Regarding the effects of sham DN on pain sensitivity, evidence is controversial. A previous study conducted in patients with chronic tension-type headache showed immediate effects of sham DN on headache frequency, which could potentially be explained by the placebo effect and/or the Hawthorne effect.[36] In the long term (6+ months), DN showed no additional benefits over sham DN in patients with neck pain.[37,38] However, the available evidence suggests greater short-term improvements when real DN is performed.[39,40] Our results suggest that both interventions induced local short-term anti-nociceptive effects at the MTrP location. However, no pain sensitivity responses at the control point location were found in the sham DN group.

Limitations

Although the results of this study are promising, some potential limitations should be recognized. First, muscle stiffness was measured only in the longitudinal plane. Since the image assessed limited fibers and MTrPs are located in taut bands, the control point could show increased stiffness. Second, the use of SWE is only moderately reliable for within-session measurements. Finally, the sample size was relatively small and further research with a larger sample is needed. Future studies should help explain the mechanisms underlying the observed improvement in local PPT in the sham DN group.

Conclusion

A single session of DN or sham DN applied to active MTrPs located in the upper trapezius muscle induced no changes in stiffness at the MTrP or control point locations. Real DN induced an immediate analgesic response at both MTrP and

control locations, while sham DN induced an immediate MTrP response. Since differences between MTrP and control point locations were found in baseline sensitvity but not stiffness, further research clarifying the clinical relevance of SWE is needed.

Contributors

JAV-C contributed to conceptualization. JAV-C, JA-G, JB-G and UV contributed to methodology. JAV-C and UV contributed to formal analysis. JAV-C, JA-G, SS-J, JB-G and UV involved in investigation. JA-G and SS-J contributed to resources. JAV-C and UV contributed to data curation. JAV-C involved in writing (original draft preparation). JAV-C and CF-d-l-P involved in writing (review and editing). JAV-C and UV contributed to visualization. JAV-C involved in supervision. JAV-C and SS-J involved in project administration. JAV-C contributed to funding acquisition. All authors read and approved the final version of the manuscript accepted for publication.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Informed consent

Informed consent was obtained from all subjects involved in the study.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of Francisco de Vitoria University (38/2021).

Study registration

This study is registered at ClinicalTrials.gov with the registration number NCT04832074.

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Data availability statement

The data that support the findings of this study are available from the first author (Juan Antonio Valera-Calero), upon reasonable request.

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