Effect of Deep Intramuscular Stimulation and Transcranial Magnetic Stimulation on Neurophysiological Biomarkers in Chronic Myofascial Pain Syndrome

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Abstract

Objective. The aim was to assess the neuromodulation techniques effects (repetitive transcranial magnetic stimulation [rTMS] and deep intramuscular stimulation therapy [DIMST]) on pain intensity, peripheral, and neurophysiological biomarkers chronic myofascial pain syndrome (MPS) patients.

Design. Randomized, double blind, factorial design, and controlled placebo-sham clinical trial.

Setting. Clinical trial in the Laboratory of Pain and Neuromodulation at Hospital de Clinicas de Porto Alegre (NCT02381171).

Subjects. We recruited women aged between 19- and 75-year old, with MPS diagnosis.

Methods. Patients were randomized into four groups: rTMS + DIMST, rTMS + sham-DIMST, sham-rTMS + DIMST, sham-rTMS + sham-DIMST; and received 10 sessions for 20 minutes each one (rTMS and DIMST). Pain was assessed by visual analogue scale (VAS); neurophysiological parameters were assessed by transcranial magnetic stimulation; biochemical parameters were: BDNF, S100β, lactate dehydrogenase, inflammatory (TNF-α, IL6, and IL10), and oxidative stress parameters.

Results. We observed the pain relief assessed by VAS immediately assessed before and after the intervention (P<0.05, F_{(1,3)}= 3.494 and F_{(1,3)}= 4.656, respectively); in the sham-rTMS + DIMST group and both three active groups in relation to sham-rTMS + sham-DIMST group, respectively. There was an increase in the MEP after rTMS + sham-DIMST (P<0.05). However, there was no change in all-peripheral parameters analyzed across the treatment (P>0.05).

Conclusion. Our findings add additional evidence about rTMS and DIMST in relieving pain in MPS.

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patients without synergistic effect. No peripheral biomarkers reflected the analgesic effect of both techniques; including those related to cellular damage. Additionally, one neurophysiological parameter (increased MEP amplitude) needs to be investigated.

Key Words. Myofascial Pain; BDNF; Oxidative Parameters

Introduction

The chronic Myofascial Pain Syndrome (MPS) is a highly prevalent problem among chronic pain complaints [1]. It is characterized by the presence of trigger points (TrPs) [2] and is linked to central and peripheral sensitization [3], suggesting a “maladaptive plasticity” condition in which the pathophysiology is not completely understood. The involvement of biological substances in the periphery may contribute to induction and maintenance of the maladaptive process, as they are capable of activating different endogenous systems: immune [4], autonomic [5], endocrine [6], and their interaction. It is interesting to note that bioactive substances were found in active TrPs, such as higher levels of inflammatory mediators, neuropeptides, catecholamines, and cytokines associated with lower pH levels; those mediators can be released and act on muscle, nerve, and connective tissue [7].

The biochemical alterations associated to MPS have been described in the TrPs; however, further serum alterations deserve more in depth research due to their diagnostic and therapeutic potential use, as is currently studied for other chronic pain conditions. For example, high levels of brain-derived neurotrophic factor (BDNF) have been found in fibromyalgia [8], and migraine attack [9] while lower levels in major depressive disorders [10]. High levels of tumor necrosis factor (TNF)-α in plasma and cerebrospinal fluid have been shown in migraines and persistently headache [11,12]. Additionally, recent studies suggest a role of oxidative stress in the pathophysiological mechanisms of fibromyalgia, tension-type headache [13], and complex regional pain syndrome [14]. Nevertheless, they have been poorly explored for MPS.

Aiming to modulate the sensitization process associated with the disease, neuromodulatory techniques that can be delivered in the peripheral or central sites, such as deep intramuscular stimulation therapy (DIMST) and repetitive transcranial magnetic stimulation (rTMS), might provide new insights. Separately, DIMST and rTMS have already demonstrated an analgesic role [15–20], and a modulator effect of peripheral mediators [21–24] in other pain conditions, and a possible transcranial magnetic stimulation (TMS)-mediated anti-oxidant effect [25,26]. Thus, we hypothesize that their association could further offer top-down (central to peripheral) and bottom-up (peripheral to central) neuromodulation, potentially enhancing the modulation of the sensitization processes that accompany MPS.

We aimed to assess the analgesic effect of the association of these neuromodulation techniques (rTMS and DIMST) in patients with chronic MPS; and to explore their underlying neurobiological mechanisms, assessing peripheral (i.e., BDNF, S100β, lactate dehydrogenase (LDH), TNF-α, interleukin (IL)-6, IL-10, superoxide dismutase (SOD), catalase activity, glutathione peroxidase (GPx), protein carbonyls, and reactive oxygen species (ROS)), and neurophysiological parameters (i.e., motor evoked potential (MEP), intracortical inhibition, intracortical facilitation (ICF), and cortical silent period (CSP4)).

Methods

The methods and results sections are reported according to the CONSORT guidelines [27].

Design Overview, Setting, and Participants

All patients provided written informed consent before participating in this randomized, double blind, factorial design, and controlled placebo-sham clinical trial. The protocol was approved by the Research Ethics Committee at the Hospital de Clínicas de Porto Alegre (HCPA) (N°. 100196 and 100276, Postgraduate Research Group at Hospital de Clínicas Porto Alegre—GPPG-HCPA; NCT02381171) and is in accordance with the of Helsinki. We recruited women aged 19 to 75 years from the general population using postings in health care units and referrals from the Chronic Pain Service at HCPA, who had a diagnosis of MPS. After committee approval, we started the data collection in February at 2012 and finished at February at 2014.

Myofascial Pain Syndrome

For inclusion, diagnoses were confirmed by an experienced clinician (>10 years of pain practice) according to the following criteria: regional pain, normal neurologic examination, TrPs, taut bands, tender points, and pain characterized as “dull,” “aching,” or “deep.” Additionally, palpable nodules, stress-exacerbated pain, decreased range of motion, and muscle ropiness was required for the diagnosis [28–30]. In addition, the patients must have suffered from an MPS limitation or disability during active and routine activities several times a week during the last three months. Their limitation was assessed using a structured questionnaire with yes/no questions about whether the patients had trouble with any of the following situations during the last three months: 1) work; 2) enjoyable activities; 3) responsibilities at home; 4) relationships; 5) personal goals; and 6) thinking clearly, problem solving, concentrating, or recall. For inclusion, patients had to have at least on positive answer for the disability questions.

Exclusion Criteria

Rheumatoid arthritis, fibromyalgia, previous surgery on the affected areas, primary radiculopathy, neurologic...
condition such as stroke or Parkinson’s disease, any systemic inflammatory disease, or being unable to come to the hospital for evaluations. Subjects were also excluded if they reported a habitual use of anti-inflammatory steroids or if they were illiterate.

Outcomes

The primary clinical outcome was pain, as assessed by visual analogue scale (VAS) and BDNF serum level; and the secondary outcomes were the peripheral biomarkers (S100β, LDH, TNF-α, IL-6, IL-10, SOD, catalase activity, GPx, protein carbonyls, and ROS) and the cortical excitability parameters (MEP, ICF, CSP, and SICI).

Sample Size

The sample size was calculated based on previous study using rTMS [31] with effect size Cohen’s (f2) of 0.28 for the dependent variable (VAS), which is considered a medium-large effect size [32]. The minimum required sample size given our analysis plan using a repeated measures correlation of 0.2 for 10 assessments and four groups for one dependent variable (VAS), 80% statistical power level and 5% error, was 44 subjects (GPower 3.1.7).

Randomization and Blinding

Participants were randomized to one of the four groups, using a stratified blocked randomization scheme and appropriate statistical Random Allocation Software. The randomization groups were 1) rTMS and DIMST; 2) rTMS and sham-DIMST; 3) sham-rTMS and DIMST; and 4) sham-rTMS and sham-DIMST. A block size of 8 and 12 was used to ensure that equal numbers of participants were randomized to the four treatment groups. Before the recruitment phase, envelopes containing the protocol materials were prepared. Each envelope was sealed and numbered sequentially and contained the allocated treatment. During the entire protocol timeline, two investigators who were not involved in patient evaluations were responsible for the blinding and randomization procedures. Other individuals who were involved in patient care were unaware of the treatment group to which the patients belonged, except those that applied the treatment. All participants were instructed not to discuss their group assignment during the treatment sessions or with the project staff collecting outcomes data, all of them were also blind to the group assignments. Independent evaluators’ blind to the group assignments were trained to apply the pain scales and cortical excitability parameters (Figure 1).

Interventions

Transcranial Magnetic Stimulation

Motor cortex excitability was assessed using TMS with a MagPro X100 (Magventure Company, Lucernemarcken, Denmark) and a figure-of-eight coil. The hot spot was marked on the scalp with a soft-tip pen. The coil was held tangentially to the scalp with the handle pointing back and away from the midline, at 45°. All participants underwent rTMS delivered at 80% resting motor threshold (rMT) intensity in a total of 10 sessions for 20 minutes each one. The coil was placed over the left primary motor cortex. For Sham rTMS treatment, a sham coil was used.

Deep Intramuscular Stimulation Therapy

We used acupuncture needles with guide tubes (Suzhou Huanqi Acupuncture Medical Appliance, 218, China) that were 40 mm in length and 0.25 mm in diameter. The needling in DIMST was applied using an electroacupuncture device (Cosmotron, São Paulo, Brazil) in the dermatomes corresponding to the nerve roots C2-C3, C3-C4, and C4-C5. A paraspinal DIMST using acupuncture needle was administered maintaining a distance from the spinous process line of 1.5 cm. All patients received 10 sessions during 20 minutes using a frequency of 2 Hz.

For the placebo-controlled condition, we used an electroacupuncture device (Cosmotron, São Paulo, Brazil), which was adjusted beforehand to prevent the current from passing through the electrodes. The electrical connection between the stimulator and the patient was broken at the output jack plug of the stimulator so that no current could pass to the patient. The patients were informed that this was a high frequency, low-intensity stimulation, and that they would most likely feel no sensation from it. The paraspinal electrodes were placed over the dermatomes, myotome, or sclerotome where the TrPs were found, and also over the main painful TrP, and the nerve stimulation unit was left in front of the patient for 20 minutes. This positioning ensured that the flashing diode that simulated the electrical stimulus was both visible and audible.

Instruments and Assessments

Demographic data and medical comorbidity were assessed using a standardized questionnaire.

All tests used in this study were validated for the Brazilian population. Each patient was submitted to all tests in the presence of a previously trained evaluator.

Parameters of Cortical Excitability

The parameters of cortical excitability were measured [20] using a MagPro X100 (Magventure Company, Denmark) and a standard figure-8 coil (Cool-B70 Butterfly) with an outer diameter of 97 mm. The coil was centered over the left motor cortex (M1 right hand) tangentially to the skull at approximately 45° angle to the sagittal plane. TMS is a non-invasive neurophysiologic
A tool that is used to assess different aspects of cortical excitability and to provide insight into the nature and localization of inhibitory and excitatory processes within cortical networks [33]. The subjects were seated in a comfortable reclining chair with their arms and hands lying relaxed on the armrests. Before starting the measurements of the parameters, we determined the optimal coil position for evoking maximal MEPs in the right first dorsal interosseous (FDI) muscle (referred to as a “motor hot spot”) using surface electromyography with Ag–AgCl cup electrodes in a belly-tendon montage. First, the rMT was defined as the stimulus intensity at which 50-\mu Vpeak-to-peak of MEP amplitude was obtained in at least 5 of 10 consecutive trials. The MEP was defined as 130% of the MT stimulus intensity at which amplitude peak-to-peak of at least 1 mV was obtained over 10 consecutive trials. The subjects were instructed to apply pressure to the dynamometer at 20% of their maximal force during a single pulse stimulus (130% MT) to the M1 hand, to elicit the cortical silent period (CSP) over 10 consecutive trials. Paired pulse measures included short intracortical inhibition (SICI) using an interstimulus interval (ISI) of 2 ms and ICF using an ISI of 12 ms. The first pulse was a subthreshold stimulus (80% MT), and the second pulse was a suprathreshold stimulus (MEP intensity). Thirty trial recordings were made in a random order having an interval of approximately 8 ms between each pulse. Paired-pulse parameters were expressed as the amount of inhibition or facilitation (ICI or \( ICF = \text{conditioned MEP/unconditioned MEP ratio} \)). We described the parameters following the checklist [34].

The FDI region is being used to evaluate the parameters of motor cortex excitability, independent of disease or condition, such as pain [35,36], obsessive–compulsive disorder [37], or depression [38]. In addition, MEPs are larger and usually have a lower threshold in distal muscles compared with proximal muscles [39]. Thus, in the present study, we used the FDI region to measure the motor cortex excitability in MPS patients.

**Pain Measurement**

The pain intensity was measured using a 10-cm VAS [40]. The VAS scores ranged from no pain (zero) to worst possible pain (10 cm). The time of the worst pain during the last 24 hours was recorded daily in the patients’ diaries.

**Biochemical Assessments**

The blood samples were collected from all of the subjects at baseline, on the fifth treatment day (after
receiving the intervention, and on the tenth day (immediately before receiving the intervention). The blood samples were centrifuged in plastic tubes for 10 minutes at 4,500 rpm at 4°C, and the serum and plasma were stored at −80°C. The erythrocytes were washed three times with saline solution (NaCl 0.9% 1:1), and were stored at −80°C in 1 mM acetic acid and 4 mM MgSO₄ solution.

BDNF, S100β, TNF-α, IL-6, IL-10

An enzyme-linked immunosorbent assay (ELISA) was performed according to the manufacturer’s instructions; BDNF serum using a ChemiKine BDNF Sandwich ELISA Kit (CYT306, Chemicon/Millipore, Billerica, MA, USA); S100β Kit assay (EZH100B-33K, Millipore, Missouri, USA) with the lower detection limit of the kit was 2.7 pg/mL; TNF-α Kit assay (KHC3011, Invitrogen, Frederick, MD); IL-6 Kit assay (DuoSet DY206, R&D Systems); IL-10 Kit assay (DuoSet DY217B, R&D Systems).

Lactate Dehydrogenase

The assay was performed using spectrophotometer kit according to the manufacturer’s instructions of Laborclin Bioliquid, the enzyme was expressed in U/L.

Oxidative Stress Parameters

The catalase activity (CAT) was determined by the evaluation of the decrement of 240-nm absorption of hydrogen peroxide (H₂O₂) according Aebi [41] and the activity was expressed as μmoles/mg of protein. The SOD activity was based on the inhibition of the superoxide radical reaction with pyrogallol [42] and expressed as USOD/mg of protein. The GPx activity was measured according Flohé and Gunzler [43] and was expressed as pmol/mg of protein. The protein oxidation by means of carbonyl group was measured according Reznick and Packer [44] and was expressed as nmol/mg of protein. ROS was performed according LeBel et al. [45] and was expressed as nmol/mg of protein. The protein was measured by Lowry method [46] and was expressed as mg.

Statistical Analysis

One-way ANOVA test or Kruskal–Wallis test for independent samples were used to analyze the continuous variables and the categorical variables were assessed by Chi-squared or Fisher’s exact tests. The outcome measures were excitability parameters (MEP, SICI, ICF, and CSP); and biochemical serum level parameters (BDNF, S100β, LDH, cytokines, and oxidative stress parameters). After first checking the assumptions of normality for the outcome measures using skewness and kurtosis tests, we conducted a group analysis by running a mixed ANOVA model in which the independent variables were time, experimental group, the interaction term time vs experimental group, and subject identification. If appropriate, we performed Bonferroni’s test for post hoc multiple comparisons to identify the differences between the groups and at each time point. We decide to run the analyses per protocol to avoid possible bias, considering that our aim was to understand the effect of interventions on physiological parameters according to intention-to-treat analysis [47]. The data were analyzed using SPSS version 20.0 (SPSS, Chicago, IL) and considered significant when P < 0.05.

Results

Patient Characteristics

The clinical and demographic characteristics of the patients are shown in Table 1. Eleven patients were allocated to rTMS + DIMST group, 12 were allocated to the rTMS + sham-DIMST group, 12 were allocated to sham-rTMS + DIMST, and 11 were allocated to sham-rTMS + sham-DIMST. Forty-four patients completed the study; one patient in the rTMS group and one patient in the DIMST withdrew due to treatment inefficacy. Baseline characteristics were similar across the four groups (all P values > 0.05) (Table 1). We did not observe serious or moderate side effects from the treatment.

Pain VAS

Pain was assessed before and after each intervention session. Raw data regarding each moment are presented in Table 2. We observed effect of treatment on Pain VAS assessed immediately before the intervention (P < 0.05, F₅,₉₃= 3.49) and time (P < 0.05, F₅,₉₃=7.55, mixed model analysis). The calculated effect size f of treatment is 0.53, with a post hoc power of 0.99. The sham-rTMS + DIMST presented lower level of pain than sham-rTMS + sham-DIMST group (Figure 2 and Table 2). In relation to time, the VAS on the first day was different from seventh, ninth, tenth, and the second day different from ninth and tenth days (P < 0.05). Additionally, there was a tendency to be different the first day from fifth (P = 0.05), sixth (P = 0.064), and eighth (P = 0.08) days.

In relation to Pain VAS assessed immediately after the intervention, we observed effect of treatment (P < 0.05, F₅,₉₃= 4.65) and time (P < 0.05, F₅,₉₃=2.68, mixed model analysis). The calculated effect size f of treatment is 0.56, with a post hoc power of 0.99. All groups presented lower level of Pain VAS than sham-rTMS + sham-DIMST. The first and second day intervention was different from the ninth day (P < 0.05) (Figure 2). However, there was no observed synergistic effect related to the association of these two techniques.
Parameters of Motor Cortical Excitability

We observed an interaction between treatment and time (\( P < 0.05 \), mixed linear models). There was an increase in the MEP after rTMS + sham-DIMST in relation to pretreatment (\( P < 0.05 \), mixed model analysis). We observed a tendency to increase after the rTMS + DIMST treatment in relation to pretreatment (\( P = 0.08 \), mixed model analysis, Figure 3).

No differences were found in the SICI, ICF, and CSP before and after treatment (\( P > 0.05 \), mixed linear models, Table 3).

Biochemical Parameters

The interventions did not change the parameter of neuroplasticity (serum levels of BDNF), neither the inflammatory markers (serum levels of TNF-\( \alpha \), IL6, and IL10) nor

Table 1 Characteristics of the study sample

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>rTMS Active and DIMST</th>
<th>rTMS Active and DIMST</th>
<th>rTMS Sham and DIMST</th>
<th>rTMS Sham and DIMST</th>
<th>( P )</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Active (n = 11)</td>
<td>Active (n = 12)</td>
<td>Sham (n = 12)</td>
<td>Sham (n = 11)</td>
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<tr>
<td>Age (years)*</td>
<td>49.18 (11.63)</td>
<td>45.83 (9.63)</td>
<td>47.25 (11.00)</td>
<td>46.73 (13.09)</td>
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<td>Body mass index*</td>
<td>25.16 (3.28)</td>
<td>26.27 (3.73)</td>
<td>25.77 (1.48)</td>
<td>26.08 (4.46)</td>
<td>0.93</td>
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<td>Education (years)*</td>
<td>15.09 (3.59)</td>
<td>11.25 (5.17)</td>
<td>11.92 (3.75)</td>
<td>13.18 (3.34)</td>
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<tr>
<td>Smoking (yes/no)†</td>
<td>0/11</td>
<td>0/12</td>
<td>1/11</td>
<td>1/10</td>
<td>0.55</td>
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<tr>
<td>Alcohol use (yes/no)†</td>
<td>7/4</td>
<td>5/7</td>
<td>5/7</td>
<td>8/3</td>
<td>0.33</td>
</tr>
<tr>
<td>Clinical comorbidity (yes/no)†</td>
<td>4/7</td>
<td>6/6</td>
<td>8/4</td>
<td>3/8</td>
<td>0.25</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4/7</td>
<td>3/9</td>
<td>4/8</td>
<td>2/9</td>
<td></td>
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<td>Hypothyroidism</td>
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<td>1/11</td>
<td>1/11</td>
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<td>Asthma</td>
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<td>0/12</td>
<td>0/12</td>
<td>0/11</td>
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</tr>
<tr>
<td>Other</td>
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<td>1/11</td>
<td>5/7</td>
<td>1/10</td>
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</tr>
<tr>
<td>Pain on VAS (cm)*</td>
<td>6.54 (1.75)</td>
<td>6.67 (2.06)</td>
<td>5.70 (3.49)</td>
<td>5.83 (3.38)</td>
<td>0.77</td>
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<td>Analgesic drug use more than three times per week during the last three months (yes/no)†</td>
<td>5/6</td>
<td>6/6</td>
<td>5/7</td>
<td>8/3</td>
<td>0.53</td>
</tr>
<tr>
<td>Pittsburgh sleep questionnaire*</td>
<td>14.91 (5.56)</td>
<td>19.17 (7.26)</td>
<td>20.09 (8.18)</td>
<td>17.18 (7.10)</td>
<td>0.33</td>
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<tr>
<td>Beck depression inventory*</td>
<td>13.64 (5.52)</td>
<td>15.83 (9.16)</td>
<td>17.73 (10.39)</td>
<td>10.55 (8.66)</td>
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<td>State-anxiety on STAI*</td>
<td>29.09 (6.70)</td>
<td>30.42 (8.39)</td>
<td>28.18 (9.23)</td>
<td>26.00 (8.36)</td>
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<td>Trait-A anxiety on STAI*</td>
<td>23.91 (7.36)</td>
<td>25.00 (7.14)</td>
<td>25.09 (8.87)</td>
<td>22.91 (6.30)</td>
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<td>Brazilian Portuguese catastrophizing scale (B-PCS)*</td>
<td>25.36 (13.34)</td>
<td>32.83 (10.09)</td>
<td>30.73 (10.87)</td>
<td>25.91 (16.10)</td>
<td>0.43</td>
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<tr>
<td>Profile of chronic pain: screen for a Brazilian population (B-PCP:S)*</td>
<td>59.09 (18.03)</td>
<td>56.50 (17.0)</td>
<td>60.59 (16.93)</td>
<td>56.32 (16.07)</td>
<td>0.92</td>
</tr>
<tr>
<td>Pain intensity reported on B-PCP-S*</td>
<td>23.36 (2.77)</td>
<td>24.83 (3.65)</td>
<td>24.14 (3.95)</td>
<td>25.32 (2.45)</td>
<td>0.53</td>
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<tr>
<td>Interference on activities reported on B-PCP:S</td>
<td>23.91 (10.27)</td>
<td>19.17 (10.04)</td>
<td>23.00 (11.21)</td>
<td>18.55 (8.73)</td>
<td>0.50</td>
</tr>
<tr>
<td>Emotional burden due pain reported on B-PCP:S</td>
<td>11.82 (6.03)</td>
<td>12.50 (7.21)</td>
<td>13.45 (5.24)</td>
<td>12.50 (7.21)</td>
<td>0.95</td>
</tr>
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<td>6/6</td>
<td>6/6</td>
<td>2/9</td>
<td>0.28</td>
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<tr>
<td>Active use of central nervous system medication (yes/no)†</td>
<td>4/7</td>
<td>5/7</td>
<td>5/7</td>
<td>1/10</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Values are given as the mean (SD) or frequency (%) (n = 46).

1) Psychotropic medication: tricyclic antidepressant, benzodiazepines, serotoninergic and/or noradrenergic antidepressant, and monoamine oxidase inhibitors; nortriptiline, amitriptiline, gabapentine, topiramate, pregabalin, opioid analgesic alone or in association and 2) Painkiller: diclofenac, dipyrone, acetaminophen, acetysalicylic acid, cyclooxygenase 2 inhibitor, or non-steroid anti-inflammatory associated with miorelaxant and/or caffeine.

†Chi-squared or Fisher’s exact test to compare frequencies.

VAS = visual analogue scale.
the oxidative stress parameters, as oxidative stress (protein carbonyls, ROS) and antioxidant enzymatic activity (SOD, catalase activity, GPx) ($P > 0.05$, mixed linear models, Table 3). Additionally, the treatments did not change the serum levels of S100$\beta$ and LDH ($P > 0.05$, mixed linear models, Table 4).

### Discussion

Our findings support the hypothesis that both neuromodulatory techniques (top-down and bottom-up) are capable of modulating the nociceptive system in MPS patients, as assessed by the clinical outcome, pain on...
VAS. Additionally, the top-down neuromodulatory technique increased the motor cortex excitability indexed by changes in the MEP amplitude. There was no evidence of increasing peripheral levels of noxious or damage response, as oxidative stress parameters, pro-inflammatory cytokines, as well as S100β and LDH. Despite the analgesic observed in the nociceptive system, there was no repercussion in the peripheral biomarkers associated with neuroplasticity (BDNF), anti-inflammatory actions (IL-10), and antioxidants systems (SOD, CAT, GPx).

In the present study, we showed that all the active interventions (rTMS, DIMST, and rTMS plus DIMST) showed reduction in pain intensity across the treatment in MPS patients, which is corroborated by data of previous studies, however in different chronic pain conditions [17,20]. For peripheral techniques, the acupuncture, electroacupuncture, dry needling, and DIMST have being studied for treating musculoskeletal pain [15–17]. The association of electrical stimulation can contribute to the analgesic effect of acupuncture [48,49], while low frequencies (2 Hz) facilitates the release of enkephalins, and high frequencies (100 Hz), release of dynorphins [50,51] and activation of descending inhibitory system [52,53]. Evidence shows that sensory inputs can produce alterations in the motor cortex excitability [54], including the acupuncture in healthy subjects [55]. However, after 10 days of treatment of DIMST, the present study there was no evidence of alteration in any parameters of the motor excitability.

Additionally, for central neuromodulatory techniques, the rTMS has being a promise in the chronic pain treatment [18], principally related to motor cortex stimulation [19,20]. The possible analgesic mechanisms of motor cortex stimulation can be related to decrease of sensorial input [56], increase of activity of periaqueductal gray [57] and restoration of intracortical gabaergic systems [20]. It is interesting to note that the rTMS plus DIMST presented analgesic effect in MPS without synergistic effect (Figure 2). Thus, we can suggest that the analgesic mechanisms of these techniques, independently of site of stimulation, may share at least in part the same pathways of action, for example descending inhibitory system. Despite the analgesic effects observed of the neuromodulatory techniques, this action cannot be reflecting in the peripheral biomarkers assessed in the present study.

Meanwhile, some pain process can be related to neurophysiological parameters assessed by TMS, for example, the enhanced MEP [58], the decreased MEP induced by experimental pain [59]. Moreover, the analgesic effect of motor cortex rTMS can be associated, at least in part, to the neurophysiological parameter of SICI [20]. In the present study, we observed changes in one neurophysiological parameter after rTMS intervention, increased MEP amplitude, which is underlying with the excitability of the corticospinal pathway [60]. As suggested from the literature, the high frequency rTMS (>5 Hz) tends to increase the motor cortex excitability [61]. As, we can suggest a possible relationship between our finding and the analgesic effect observed after this intervention.

It is known that the BDNF serum levels derived 75% from the central nervous system [62], reflecting the central levels [63], and this neurotrophin is linked to the central sensitization process [64–67]. However, the effect of TMS on BDNF serum is still controversy, as decreased BDNF levels after rTMS [68], no effect on BDNF after acute TMS [69] and rTMS [21,70,71].

**Figure 3** MEP assessed before and after intervention. Values are given as mean (standard error of the mean). *Significant difference after from before (P < 0.05, linear mixed model analysis).

**Table 3** Parameters of cortical excitability of the study sample

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Intracortical Facilitation</th>
<th>Short Intracortical Inhibition</th>
<th>Cortical Silent Period (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Final</td>
<td>Baseline</td>
</tr>
<tr>
<td>rTMS + DIMST</td>
<td>1.04 (0.18)</td>
<td>1.05 (0.19)</td>
<td>0.33 (0.14)</td>
</tr>
<tr>
<td>rTMS + Sham-DIMST</td>
<td>1.41 (0.71)</td>
<td>1.20 (0.36)</td>
<td>0.54 (0.85)</td>
</tr>
<tr>
<td>Sham-rTMS + DIMST</td>
<td>1.15 (0.29)</td>
<td>1.26 (0.16)</td>
<td>0.28 (0.16)</td>
</tr>
<tr>
<td>Sham-rTMS + Sham-DIMST</td>
<td>1.07 (0.19)</td>
<td>1.09 (0.26)</td>
<td>0.34 (0.14)</td>
</tr>
</tbody>
</table>

Values are given as the mean (SD) (n = 46).
No differences were found (P > 0.05).
## Table 4  Biochemical parameters of the study sample

<table>
<thead>
<tr>
<th></th>
<th>rTMS + DIMST</th>
<th>rTMS + Sham-DIMST</th>
<th>Sham-rTMS + DIMST</th>
<th>Sham-rTMS + Sham-DIMST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Day 5</td>
<td>Day 10</td>
<td>Baseline</td>
</tr>
<tr>
<td>BDNF (ng/mL)</td>
<td>21.86</td>
<td>24.27</td>
<td>25.09</td>
<td>22.32</td>
</tr>
<tr>
<td></td>
<td>(15.00)</td>
<td>(14.38)</td>
<td>(18.08)</td>
<td>(33.30)</td>
</tr>
<tr>
<td>S100β (pg/mL)</td>
<td>17.40</td>
<td>14.32</td>
<td>15.46</td>
<td>13.49</td>
</tr>
<tr>
<td></td>
<td>(11.09)</td>
<td>(6.07)</td>
<td>(8.50)</td>
<td>(11.45)</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>28.09</td>
<td>27.81</td>
<td>28.15</td>
<td>28.18</td>
</tr>
<tr>
<td></td>
<td>(2.89)</td>
<td>(7.22)</td>
<td>(9.98)</td>
<td>(2.71)</td>
</tr>
<tr>
<td>IL10 (pg/mL)</td>
<td>0.55</td>
<td>0.58</td>
<td>0.44</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>(0.41)</td>
<td>(0.56)</td>
<td>(0.59)</td>
<td>(2.67)</td>
</tr>
<tr>
<td>IL6 (pg/mL)</td>
<td>0.41</td>
<td>0.43</td>
<td>0.38</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>(0.36)</td>
<td>(0.45)</td>
<td>(0.39)</td>
<td>(0.32)</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>87.21</td>
<td>101.28</td>
<td>93.40</td>
<td>87.22</td>
</tr>
<tr>
<td></td>
<td>(51.21)</td>
<td>(46.98)</td>
<td>(23.92)</td>
<td>(36.86)</td>
</tr>
<tr>
<td>GPX (μmoles/mg of protein)</td>
<td>0.45</td>
<td>0.57</td>
<td>0.48</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>(0.98)</td>
<td>(0.59)</td>
<td>(1.22)</td>
<td>(0.82)</td>
</tr>
<tr>
<td>SOD (USOD/mg of protein)</td>
<td>97.55</td>
<td>111.25</td>
<td>115.05</td>
<td>70.64</td>
</tr>
<tr>
<td></td>
<td>(82.09)</td>
<td>(108.39)</td>
<td>(131.98)</td>
<td>(99.98)</td>
</tr>
<tr>
<td>CAT (μmoles/mg of protein)</td>
<td>717.30</td>
<td>619.7</td>
<td>808.25</td>
<td>753.95</td>
</tr>
<tr>
<td></td>
<td>(491.63)</td>
<td>(491.35)</td>
<td>(501.20)</td>
<td>(810.80)</td>
</tr>
<tr>
<td>CAR (nmol/mg of protein)</td>
<td>112.15</td>
<td>108.65</td>
<td>105.50</td>
<td>113.65</td>
</tr>
<tr>
<td></td>
<td>(30.00)</td>
<td>(23.55)</td>
<td>(21.43)</td>
<td>(33.55)</td>
</tr>
<tr>
<td>ROS (nmol/mg of protein)</td>
<td>0.83</td>
<td>1.02</td>
<td>1.18</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>(0.57)</td>
<td>(0.89)</td>
<td>(0.84)</td>
<td>(0.42)</td>
</tr>
</tbody>
</table>

Values are given as the mean (SD) (n = 46).
No differences were found (P > 0.05).
LDH = lactate dehydrogenase; GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase activity; CAR = protein carbonyls; ROS = reactive oxygen species.
increased BDNF serum levels after rTMS [24,72]. These differences showed in the literature can be associated to the polymorphism of BDNF (Val66Met) [73], and mainly related to parameters used of rTMS technique. In the present study, no difference was found in the BDNF serum levels suggesting there was not alteration in the central induced by neuromodulatory techniques exposure at least with stimulation site and time used.

The main adverse effects of acupuncture and dry needling are associated with local hemorrhage and bleeding, and vegetative symptoms [74–76]; while the TMS can commonly induce pain and discomfort, headache and neck pain [77,78], as well as syncope and rarely seizure [79]. Additionally, after intense noxious stimulation the central sensitization process may be initiated and maintained by substances as substance P, TNF-α, and BDNF [80,81], IL-1β and IL-6 [82]. Additionally, IL-6, TNF-α, and BDNF promote the release of excitatory neurotransmitters, as well decreasing the inhibitory neurotransmission [81]. It is interesting to note that the patients with central sensitivity syndrome (chronic tension-type headache, fibromyalgia, and chronic MPS patients) presented higher serum levels of BDNF and TNF-α and lower serum levels of IL-10 and IL-6 than control sample and the non-central sensitivity syndrome (osteoarthritis and endometriosis) [83].

Furthermore, the S100β is highly expressed in astrocytes [84], and its high serum levels were demonstrated in adults with both medical and traumatic causes of brain injury [85–87]; while the LDH reflects cell damage [88] and its high levels is associated with oxidative stress [89]. Moreover, recent studies have been showed that electromagnetic fields are potential exogenous source of oxidants [90–92], which can be involved with central sensitization [93,94].

Additionally, no alterations were observed in the peripheral levels in antioxidant enzymatic activities (SOD, catalase activity, GPx), neither IL-10 cytokine. The scavengers of ROS are being associated with relieving persistent pain [95,96], while it is shown that IL-10 can inhibit the production of other cytokines as TNF-α, IL-1, IL-6, and IL-8 in vitro [97,98]. Interesting to note that, if these neuromodulatory techniques did not contribute to increase or decrease these parameters linked to benefits to the organism in the peripheral site.

Our study had certain limitations. First, the results found in the present study could be confounded by other variables, such as the medication used (i.e., psychotropic medication or painkiller) or psychiatric conditions; regardless none of these variables were retained in the models used. Second, the cortical excitability parameters were analyzed after the last session of intervention because the feasibility of the study, however it can be confounded our findings. Third, the use of TMS for neurophysiological assessments involved the evaluation of neurotransmitter system activity in an indirect manner, and it has been shown to have relatively low specificity.

Fourth, all biochemical parameters were assessed in peripheral sample. Fifth, although the statistical analysis considers the multiple comparisons we cannot discard the error type II.

In summary, our findings add supplementary evidence about rTMS and DIMST in pain relieving in patients with MPS. In addition, the neurophysiological changed after rTMS was an increased in the MEP amplitude. However, it is important to note that these treatments did not alter any of the markers evaluated for cellular damage suggesting that these techniques are safe, at least, concerning the parameters evaluated in this study.

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