Brazilian Journal of Anesthesiology 2023;73(4): 409—417

Sociedade Brasileira de Anestesiologia

Brazilian Journal of ANESTHESIOLOGY

ORIGINAL INVESTIGATION

Association of low-dose naltrexone and transcranial direct current stimulation in fibromyalgia: a randomized, double-blinded, parallel clinical trial

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Received 17 January 2022; accepted 9 August 2022
Available online 18 August 2022

KEYWORDS
Fibromyalgia;
Naltrexone;
Pain;
Transcranial direct current stimulation

Abstract

Introduction: Fibromyalgia is a complex, generalized, and diffuse chronic musculoskeletal pain. Pharmacological approaches are widely used to relieve pain and increase quality of life. Low-Dose Naltrexone (LDN) was shown to increase the nociceptive threshold in patients with fibromyalgia. Transcranial Direct Current Stimulation (tDCS) is effective for pain management.

Objective: The purpose of this study was to evaluate the analgesic and neuromodulatory effects of a combination of LDN and tDCS in patients with fibromyalgia.

Methods: This was a randomized, double-blinded, parallel, placebo/*sham*-controlled trial (NCT04502251; RBR-7HK8N) in which 86 women with fibromyalgia were included, and written informed consent was obtained from them. The patients were allocated into four groups: LDN + tDCS (n = 21), LDN + tDCS *Sham* (n = 22), placebo + tDCS (n = 22), and placebo+tDCS *Sham* (n = 21). The LDN or placebo (p.o.) intervention lasted 26 days; in the last five sessions, tDCS was applied (sham or active, 20 min, 2 mA). The following categories were assessed: sociodemographic, Visual Analog Pain Scale (VAS), Pain Catastrophizing Scale (PCS), State-Trait Anxiety Inventory (STAI), Fibromyalgia Impact Questionnaire (FIQ), Beck Depression Inventory (BDI-II),

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https://doi.org/10.1016/j.bjane.2022.08.003

Profile of Chronic Pain Scale (PCP:S), Pain Pressure Threshold (PPT), and Conditioned Pain Modulation (CPM). Blood samples were collected to analyze BDNF serum levels.
Results: At baseline, no significant difference was found regarding all measurements. VAS pain was significantly reduced in the LDN + tDCS ($p = 0.010$), LDN + tDCS *Sham* ($p = 0.001$), and placebo+tDCS *Sham* ($p = 0.009$) groups. In the PCP:S, the LDN+tDCS group showed reduced pain frequency and intensity ($p = 0.001$), effect of pain on activities ($p = 0.014$) and emotions ($p = 0.008$). Depressive symptoms reduced after all active interventions ($p > 0.001$).
Conclusion: Combined LDN+tDCS has possible benefits in reducing pain frequency and intensity; however, a placebo effect was observed in pain using VAS, and further studies should be performed to analyze the possible association.

Introduction

Fibromyalgia is a chronic musculoskeletal pain syndrome that manifests as fatigue, morning stiffness, sleep and humor disturbances, and cognitive and memory impairment associated with other clinical symptoms such as anxiety, depression, and pain catastrophizing thought.[1] Besides resulting in impairment in quality of life, patients also show emotional reactions such as anger, depression, anxiety, loneliness, and fear.[1] These negative emotions result in an increase in pain sensitivity, resulting in a catastrophizing thought as a non-adaptive response to pain, which is one of the factors that contribute to chronic pain syndrome.[2]

Low pain threshold, high levels of anxiety, exacerbated fear, and hypervigilance can be associated with cortical dysfunctions related to afferent pathway abnormalities and sensitized cortical processes, which result in impairment of sensory processing in the brain, causing pain chronicity.[3] During the sensitization process, pain perception is amplified in the Central Nervous System (CNS), which results in a continuing pain experience with no nociceptive peripheral stimuli,[4] including psychological suffering, sleep disturbances, allodynia, and hyperalgesia.[5]

This syndrome mainly affects women of reproductive age, with a global prevalence of 2.4% to 6.8%.[6] Diagnosis is clinical because there are no biomarkers or imaging exams that provide evidence of the syndrome.[7] In 2016, the American College of Rheumatology (ACR) established the criteria for fibromyalgia diagnosis: generalized pain in at least four regions, symptoms present for at least 3-months, Generalized Pain Index (GPI) equal to or higher than 7, and severity of symptoms equal or higher than 5.[8]

New drug therapies are needed to control pain, reduce adverse effects, and increase quality of life. In this way, naltrexone, at lower doses than usual, has recently emerged as a potential agent for chronic pain management and is a possible therapeutic option for the treatment of fibromyalgia.[9]

Naltrexone is a pure opioid antagonist that acts on opioid and non-opioid receptors. Naltrexone active metabolites are reversible competitive antagonists of μ-opioids and κ-opioid receptors, with a higher affinity for μ-opioids. However, kappa receptor activation induces anti-inflammatory effects, decreasing IL-6 levels and neutrophil migration.[10] Another potential mechanism for the use of Low-Dose Naltrexone (LDN) is the antagonism of non-opioid receptors, such as Toll-like Receptor-4 (TLR4)[11] found in macrophages. TLR4 blockade inhibits the release of proinflammatory cytokines, substance P, nitric oxide, excitatory amino acids, and Tumor Necrosis Factor (TNF) leading to the downregulation of chemokines and adhesion molecule receptor expression.[12] Randomized clinical trials using 4.5 mg naltrexone have been conducted in Crohn's disease, multiple sclerosis, fibromyalgia, and HIV infection, in which evidence shows efficacy and low toxicity.[10]

Noninvasive brain stimulation techniques have emerged in global scenarios as a treatment option for chronic pain. Transcranial Direct Current Stimulation (tDCS)[13,14] is a potential treatment option because of its safety, portability, relative cost, and ease of use. It modulates the resting membrane potential.[15] A previous review/meta-analysis showed that repeated tDCS decreases pain levels in patients with fibromyalgia.[16] In addition, a recent meta-analysis of data from 8 controlled trials provided tentative evidence of pain reduction after active tDCS.[17] Altogether, it is important to note that patients with fibromyalgia are not drug-free, and the potential synergistic effect between pharmacological and non-pharmacological treatments may present an optimal response.

Therefore, this study aimed to investigate the analgesic and neuromodulatory effects of previous treatment with LDN combined with anodal tDCS in women with fibromyalgia. The secondary objective was to evaluate the effects of psychophysiological measures and peripheral Brain-Derived Neurotrophic Factor (BDNF) levels.

Methods

Study design

This was a randomized, double-blind, parallel, controlled with placebo and *sham* stimulation, clinical trial. This study was conducted from August 2018 to July 2019 at La Salle Saúde, Canoas/RS, Brazil. This study was performed in accordance with the Declaration of Helsinki, approved by the La Salle University Ethics Committee (CAAE 0005317.5.0000.5307), registered on Clinical Trials under the number NCT04502251 (https://clinicaltrials.gov), and registered in the Registro Brasileiro de Ensaios Clínicos (ReBEC) platform (RBR-7HK8N# - www.ensaiosclinicos.gov.br).

Population

All participants signed an informed consent form. The inclusion criteria were as follows: women aged 18—65 years, confirmed diagnosis of fibromyalgia according to the 2016 ACR criteria, capable to read and write, pain higher than 6 on the Visual Analog Scale (VAS) in the past 3 months, and chronic stable treatment in the past 3 months.

The exclusion criteria were as follows: use of opioid drugs, pregnancy or not using contraception methods, history of alcohol or drug abuse in the past six months, history of neurological pathologies, arrhythmia history, history of use of drugs that might change vascular response, history of head trauma, history of neurosurgery, decompensated systemic diseases or chronic inflammatory diseases (lupus, rheumatoid arthritis, Sjogren syndrome, Reiter syndrome), history of non-compensated hypothyroidism, and personal history of cancer.

Interventions

According to randomization, each participant received 21 days of low-dose naltrexone (4.5 mg) or placebo followed by 5 days of the drug combined with anodal tDCS (active or *sham*). The timelines of the intervention and measurements are presented in Figure 1.

Low-dose naltrexone (LDN), produced by a manipulation pharmacy in a 4.5 mg daily dose, was administered orally for 26 days. The placebo presented the same format, size, and color as the LDN capsules; however, starch was used as the excipient.

Transcranial Direct Current Stimulation (tDCS): an anodal electrode was placed on the scalp above the primary motor cortex (M1) contralateral to the dominant cortex. A cathodal electrode was placed in the contralateral supraorbital area. The current used was 2mA for 20 min. A battery stimulator with a constant current was used (tDCS device, TCT Research, 1 × 1).[13,18] Five stimulation sessions were performed, according to previous fibromyalgia studies.[16] *Sham*-tDCS stimulation consisted of an active current for 30s.

Sample size calculation

Sample size calculation was based on previous studies using tDCS in the M1 cortex for pain treatment in fibromyalgia.[19] Based on these data, tDCS was estimated to have a Cohen's *f* effect of 0.37. To reach a power of 80% (β = 0.20) and maintain a statistical significance level alpha of 0.05, 21 patients were required in each arm. With a 10% of total loss, there were 92 patients in total.

Randomization and blinding

Before the recruitment phase, a randomization table was generated using a website (seadenvelope.com), creating a randomization list in blocks of 8. Codes were placed in separately sealed brown envelopes, and the patients were allocated into four groups (Fig. 2). The researcher who applied the simulation and the researcher who applied the questionnaires and pain tests were blinded, and a third person set up the device. Blinding was maintained in all the study phases. To evaluate tDCS blinding, at the end of the five sessions, the patients were questioned about the intervention they believed they received.

Measurements

The primary outcome of this study was measured using the Visual Analog Scale (VAS). Baseline and demographic outcomes were measured using the sociodemographic questionnaire. Quality of life (measured using the Fibromyalgia Impact Questionnaire [FIQ]), depressive symptoms (measured by the Beck Depression Inventory-BDI-II), anxiety symptoms (measured by the State-Trait Anxiety Inventory-STAI), pain catastrophizing thought (measured by the Pain Catastrophizing Scale [PCS]), pain functional impact (measured by the Profile of Chronic Pain Scale [PCP: S]), and adverse effects (LDN and tDCS) were considered secondary outcomes.

Pain measurements were as follows: Pain Pressure Threshold (PPT) was measured using an electronic algometer applied to the right forearm, and patients reported the first pain sensation (minimum pain) and maximum pain. An electronic algometer (JTech Medical Industries) was used. The device consisted of a 1-cm^2 hard-rubber probe, which was applied over all the tender points. The average values of the PPT in $kgf.cm^{-2}$ ($lb.cm^{-2}$) for three successive readings were obtained at intervals of 3—5 min and used as the outcomes. On using Conditioned Pain Modulation (CPM), with an algometer (PPT task), patients reported a pain score of 6 on

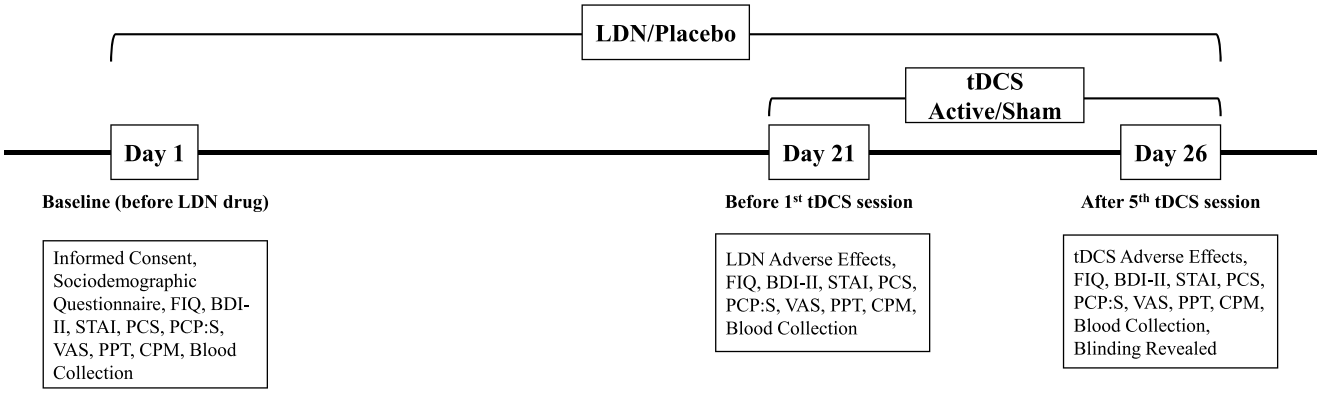

Figure 1 Study timeline.

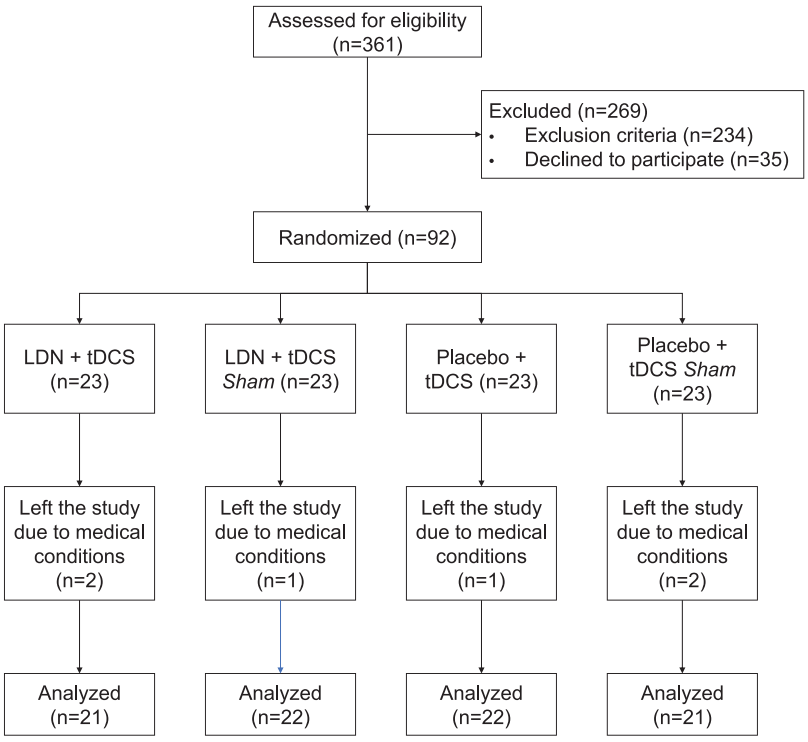

Figure 2 Study Flowchart.

the VAS. This pain level was applied to the right forearm, while the left forearm (non-dominant hand) was submerged in water from 0°C to 1.5°C; after 30 s, patients reported pain in each arm. The CPM formula was right forearm VAS, 6.

Blood was collected and centrifuged, and the supernatant was used for BDNF analysis using ELISA, according to the manufacturer's instructions. Serum BDNF levels were measured using a single method. Blood serum was collected at three time points: on the first day before LDN treatment, before the first tDCS session, and after the last tDCS session.

Statistical analysis

Data are tabulated in red cap. Continuous variables are described as mean ± standard error, while categorical variables are described as percentages. The Shapiro-Wilk test was used for sample distribution, considering a normal distribution when $p > 0.05$. To evaluate baseline data, one-way ANOVA or Kruskal-Wallis test was used for continuous variables, while Fisher's exact test and chi-square test were used for categorical data. The Friedman test followed by the Wilcoxon post-hoc test was used to analyze the effects of treatment over time between groups. Statistical significance was set at $p < 0.05$. Data were analyzed using SPSS (version 20.0; SPSS, Chicago, IL, USA).

Results

Baseline data for the sample are shown in Table 1. No statistically significant differences were found between the groups.

Table 2 presents results from days 1 (baseline), 21, and 26. Visual Analogue Scale (VAS) for pain presented a significant reduction from day 1 to day 26 in the following groups: LDN + tDCS ($p = 0.010$), LDN+tDCS *Sham* ($p = 0.001$), and placebo + tDCS *Sham* ($p = 0.011$). Besides that, the LDN +tDCS *Sham* group also presented a significant reduction on comparing day 26 to day 21.

The Profile of Chronic Pain Scale (PCP:S) enabled us to observe that patients who received active association presented a significant reduction in the frequency and intensity of pain ($p = 0.001$), on comparing day 26 to day 1. Moreover, groups that received LDN presented a significant reduction in interference in activities (LDN + tDCS, $p = 0.014$; LDN + tDCS *Sham*, $p = 0.008$), on comparing day 26 to day 1. Regarding interference in emotions, only the associated group presented a significant reduction over time ($p = 0.008$) (Table 2). Figure 3 presents the data analysis from BDNF performed on days 1, 21, and 26, comparing each value per group. It is possible to identify a significant reduction in BDNF levels in the LDN + tDCS *Sham* group ($p = 0.025$), when LDN was used individually. In addition, it was possible to visualize a significant reduction in the placebo + tDCS group ($p = 0.002$) after the last tDCS intervention (day 26).

Regarding the impact of Fibromyalgia on Quality of life (FIQ), Table 3 shows that there was a significant reduction in the LDN + tDCS group ($p < 0.05$) on comparing day 26 to day 1 in terms of overall impact and function. Regarding the symptoms on the FIQ scale, all groups showed a significant reduction ($p < 0.05$).

In addition, it was possible to observe an improvement in depressive symptoms (BDI-II), in which the group that received LDN+tDCS had significant improvements on days 21 and 26 compared to day 1 ($p < 0.001$). Groups that received active intervention showed a significant reduction from day 26 to day 1 ($p = 0.001$) (Table 3).

Table 1 Sociodemographic profile.

	LDN+tDCS	LDN + tDCS Sham	Placebo+tDCS	Placebo + tDCS Sham	*p*-value
Age (y)	49.74 ± 1.97	48.09 ± 1.56	50.57 ± 2.23	48.95 ± 2.08	0.800[a]
Scholarship (y)	10.00 ± 0.53	11.55 ± 0.99	13.00 ± 0.92	11.95 ± 0.83	0.097[a]
BMI (kg.m^{-2})	27.44 ± 0.88	30.08 ± 1.30	28.37 ± 1.08	27.37 ± 0.87	0.236[a]
Use of Alcohol					0.465[b]
Yes	33.3%	13.6%	18.2%	14.3%	
No	66.7%	86.4%	81.8%	85.7%	
Smoking					0.744[b]
Yes	19%	18.2%	9.1%	9.5%	
No	81%	81.8%	90.9%	90.5%	
Use of medicine					
Tricyclic AD	23.8%	18.2%	22.7%	23.8%	0.953[b]
Serotonergic AD	33.3%	27.3%	18.2%	14.3%	0.523[b]
MAO Inhibitor	0%	4.5%	0%	0%	1.000[b]
Antipsychotic	0%	0%	4.5%	0%	1.000[b]
Anxiolytic	19%	4.5%	9.1%	23.8%	0.302[b]
Carbamazepine	0%	4.5%	0%	0%	0.948[b]
Valproic Acid	0%	0%	0%	4.8%	0.500[b]

AD, Antidepressive; BMI, Body Mass Index; LDN, Low-Dose of Naltrexone; MAO, Mono-Amino Oxidase.
[a] One-Way ANOVA — Data expressed as mean ± standard error.
[b] Fisher's Exact Test — Data expressed as percentage.

Anxiety was evaluated using the State-Trait Anxiety Index (STAI), and it was possible to observe a significant reduction in the state domain from day 21 to day 1 in the group that received only LDN (p = 0.026). The trait domain showed a significant reduction from day 26 to day 1 in the LDN + tDCS group (p = 0.003) (Table 3).

Regarding pain catastrophizing, a significant reduction was observed from day 26 to day 1 in the LDN + tDCS group (p = 0.027), which might be related to a possible reduction in pain levels. In addition, it is important to note that the placebo + tDCS *Sham* group showed a significant reduction in total catastrophism (p = 0.032). The hopelessness domain presented similar results as total catastrophism, in which the group that received both interventions and the group that received both placebo interventions had a significant reduction (p = 0.029 and p = 0.003, respectively) (Table 3).

For tDCS related adverse effects, the tDCS group presented a higher frequency of tingling, itching, and blushing than the *Sham* group ($p < 0.05$). Headache, neck ache, scalp pain, burning sensation, sleepiness, and acute mood changes did not differ between the groups ($p > 0.05$). In LDN adverse effects, there was no significant difference among the groups when the adverse effects (nausea, blurred vision, headache, sleepiness, difficulty in concentrating, and acute mood change) were analyzed ($p > 0.05$).

Discussion

To date, there has been no consensus on a specific treatment for fibromyalgia; however, pharmacological (antidepressant and anticonvulsant drugs) and non-pharmacological approaches (exercise, acupuncture) have been used. In this study, we tested this approach using LDN combined with tDCS to treat pain and other symptoms. It is interesting to point out that the combination of LDN and tDCS was able to decrease pain on VAS, decreased frequency and intensity of pain, decreased interference in activities and emotions on PCP, and the three domains on FIQ. On the other hand, the placebo group (placebo + tDCS *Sham)* was able to decrease pain on VAS, but only the symptoms domain on FIQ and PCS (total and hopelessness).

In addition, new drugs such as Low-Dose Naltrexone (LDN) have been investigated for the treatment of chronic inflammatory diseases.[11] A previous pilot study showed that naltrexone (4.5 mg) decreased auto-related symptoms, particularly pain and fatigue, in women with fibromyalgia.[20] A randomized, double-blinded, clinical trial with 31 women with fibromyalgia, with the same dose (4.5 mg), showed significant reduction in pain and severity of symptoms (humor and quality of life).[21]

In addition, the potential analgesic effect of Noninvasive Brain Stimulation (NIBS) has been investigated. A recent study showed that ten tDCS sessions applied to the M1 cortex promoted pain relief and increased humor in patients with fibromyalgia.[22] A study performed by Fregni et al. (2006)[18] with 32 patients with fibromyalgia, randomized between M1, dorsolateral prefrontal cortex (DLPFC), and *Sham*, for 20 minutes for 5 consecutive days, showed significant pain reduction in groups that received M1 stimulation. In this study, there was no pain reduction in the VAS in the group that received tDCS only (placebo + tDCS). However, after 21 days of LDN/placebo, five sessions of anodal tDCS were added to the last five days of LDN in women with fibromyalgia, and some beneficial results were found in this association, such as reduction in pain frequency and intensity, and interference in activities and emotions. We observed a significant reduction in pain levels in the groups that received LDN (LDN + tDCS and LDN + tDCS *Sham*) and in the placebo + tDCS *Sham* group. It is important to note that the association (LDN + tDCS) was not superior to the group that received only the drug (LDN + tDCS *Sham*), showing that the association may not be as beneficial as the drug used separately.

Table 2 Sample pain profile.

	Day 1	Day 21	Day 26	*p*-value	Effect size
VAS					
LDN + tDCS	7.05 ± 0.34	5.52 ± 0.55	5.10 ± 0.61[a]	**0.010**[c]	**3.948**
LDN + tDCS *Sham*	6.67 ± 0.32	6.12 ± 0.44	4.67 ± 0.58[a,b]	**0.001**[c]	**4.269**
Placebo + tDCS	6.14 ± 0.33	5.50 ± 0.49	4.41 ± 0.55	0.090	–
Placebo + tDCS *Sham*	6.23 ± 0.35	5.20 ± 0.45[a]	5.00 ± 0.59	**0.011**[c]	**2.555**
PPT – Minimum Pain					
LDN + tDCS	0.88 ± 0.15	0.74 ± 0.10	0.98 ± 0.12	0.068	–
LDN + tDCS *Sham*	0.78 ± 0.09	0.72 ± 0.91	0.94 ± 0.10	0.170	–
Placebo + tDCS	0.90 ± 0.11	0.90 ± 0.12	0.95 ± 0.13	0.955	–
Placebo + tDCS *Sham*	1.02 ± 0.14	0.74 ± 0.11	0.98 ± 0.15	0.386	–
PPT – Maximum Pain					
LDN + tDCS	2.74 + 0.40	2.62 + 0.48	3.20 + 0.42	0.919	–
LDN + tDCS *Sham*	2.99 ± 0.34	2.56 ± 0.24	2.95 ± 0.34	0.244	–
Placebo + tDCS	3.70 ± 0.51	2.96 ± 0.37	3.16 ± 0.43	0.083	–
Placebo + tDCS *Sham*	3.02 ± 0.39	2.78 ± 0.44	2.83 ± 0.49	0.140	–
CPM					
LDN + tDCS	-0.57 ± 0.74	-1.02 ± 0.69	-0.02 ± 0.61	0.589	–
LDN + tDCS *Sham*	-1.22 ± 0.56	-0.22 ± 0.50	0.41 ± 0.54	0.129	–
Placebo + tDCS	-0.68 ± 0.47	-1.18 ± 0.55	-0.22 ± 0.56	0.477	–
Placebo + tDCS *Sham*	-1.23 ± 0.69	-0.28 ± 0.59	-0.23 ± 0.70	0.193	–
PCP:S – Frequency and Intensity					
LDN + tDCS	26.80 ± 0.44	26.04 ± 0.50	24.78 ± 0.55[a]	**0.001**[c]	**4.055**
LDN + tDCS *Sham*	25.40 ± 0.42	24.78 ± 0.65	24.02 ± 0.91	0.559	–
Placebo + tDCS	25.88 ± 0.56	25.38 ± 0.56	25.77 ± 0.46	0.784	–
Placebo + tDCS *Sham*	25.65 ± 0.69	24.92 ± 0.72	24.30 ± 0.95	0.069	–
PCP:S – Interference in Activities					
LDN + tDCS	28.61 ± 1.15	23.52 ± 1.65	22.74 ± 2.14[a]	**0.014**[c]	**3.417**
LDN + tDCS *Sham*	28.09 ± 1.56	25.28 ± 1.93[a]	25.76 ± 1.99[a]	**0.008**[c]	**1.305**
Placebo + tDCS	26.36 ± 1.49	25.65 ± 1.32	25.00 ± 1.49	0.387	–
Placebo + tDCS *Sham*	25.40 ± 1.99	23.65 ± 2.11	22.15 ± 2.24	0.432	–
PCP:S – Interference in Emotions					
LDN + tDCS	16.38 ± 0.99	13.61 ± 1.11[a]	13.23 ± 1.34[a]	**0.008**[c]	**2.673**
LDN + tDCS *Sham*	17.31 ± 0.85	15.95 ± 1.21	14.90 ± 1.32	0.080	–
Placebo + tDCS	15.90 ± 1.22	14.68 ± 1.25	14.09 ± 1.26	0.084	–
Placebo + tDCS *Sham*	15.71 ± 1.05	14.61 ± 1.18	14.38 ± 1.32	0.267	–

CPM, Conditioned Pain Modulation; PCP:S, Profile of Chronic Pain Scale; PPT, Pain Pressure Threshold; VAS, Visual Analogue Scale.
Data presented as mean ± standard error. Friedman Test.
[a] Different from Day 1.
[b] Different from Day 21.
[c] Significant difference.

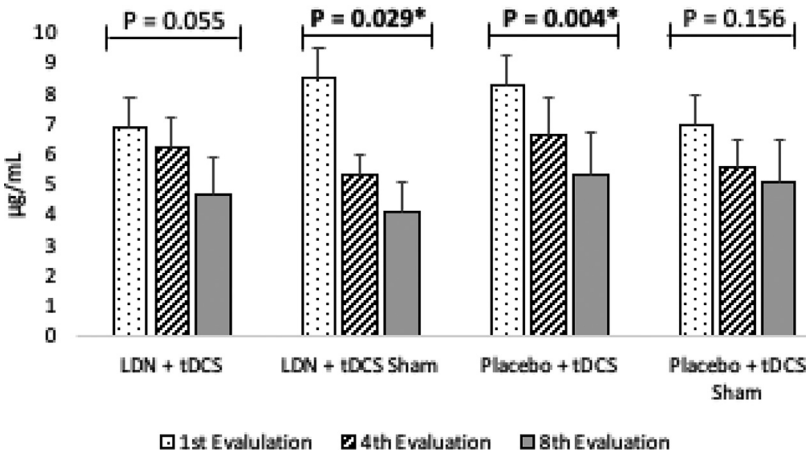

Figure 3 BDNF serum levels analysis during time. Friedman test. Data expressed as mean ± standard error.

Table 3 Data from questionnaires.

	Day 1	Day 21	Day 26	*p*-value	Effect size
FIQ-Function					
LDN + tDCS	15.88 ± 1.53	11.67 ± 0.97[a]	12.67 ± 1.06	**0.005**[c]	**3.286**
LDN + tDCS *Sham*	13.30 ± 1.38	14.00 ± 1.87	13.89 ± 1.33	0.625	-
Placebo + tDCS	15.02 ± 1.37	13.78 ± 1.30	13.82 ± 1.39	0.403	–
Placebo + tDCS *Sham*	12.95 ± 1.21	13.79 ± 1.06	13.61 ± 1.07	0.918	–
FIQ-Overall Impact					
LDN + tDCS	6.16 ± 0.25	5.85 ± 0.24	5.20 ± 0.23[a]	**0.004**[c]	**3.996**
LDN + tDCS *Sham*	5.80 ± 0.31	5.23 ± 0.26	5.20 ± 0.27	0.112	–
Placebo + tDCS	5.23 ± 0.29	5.17 ± 0.22	4.69 ± 0.18	0.304	–
Placebo + tDCS *Sham*	6.04 ± 0.23	5.43 ± 0.25	5.28 ± 0.26	0.054	–
FIQ-Symptoms					
LDN + tDCS	8.01 ± 0.33	6.62 ± 0.46	5.94 ± 0.47[a]	**<0.001**[c]	**5.097**
LDN + tDCS *Sham*	7.96 ± 0.28	6.50 ± 0.42	6.00 ± 0.43[a]	**0.003**[c]	**5.401**
Placebo + tDCS	7.68 ± 0.33	6.20 ± 0.45	5.40 ± 0.47[a]	**0.003**[c]	**4.630**
Placebo + tDCS *Sham*	7.73 ± 0.36	6.60 ± 0.40	5.97 ± 0.49[a]	**0.003**[c]	**4.093**
BDI-II					
LDN + tDCS	24.38 ± 2.18	20.05 ± 1.92	17.33 ± 1.76[a]	**<0.001**[c]	**3.558**
LDN + tDCS *Sham*	28.86 ± 2.29	23.29 ± 2.52	20.90 ± 2.67[a]	**0.001**[c]	**3.200**
Placebo + tDCS	24.18 ± 1.82	21.36 ± 2.29	17.86 ± 2.45[a,b]	**0.001**[c]	**2.928**
Placebo + tDCS *Sham*	22.20 ± 2.35	19.25 ± 2.09	16.40 ± 2.32	0.086	–
STAI – State					
LDN + tDCS	26.33 ± 0.54	27.50 ± 0.72	27.19 ± 0.70	0.590	–
LDN + tDCS *Sham*	27.86 ± 0.91	26.43 ± 0.86[a]	27.71 ± 1.00	**0.026**[c]	**1.615**
Placebo + tDCS	27.82 ± 0.76	27.68 ± 1.02	28.27 ± 1.00	1.000	–
Placebo + tDCS *Sham*	27.40 ± 0.94	28.10 ± 1.21	27.25 ± 0.71	0.607	–
STAI – Trait					
LDN + tDCS	24.86 ± 0.65	23.52 ± 0.75	21.86 ± 1.26[a]	**0.003**[c]	**2.992**
LDN + tDCS *Sham*	25.67 ± 0.63	25.24 ± 0.75	25.43 ± 0.61	0.607	–
Placebo + tDCS	25.45 ± 0.66	24.64 ± 0.65	24.27 ± 0.72	0.277	–
Placebo + tDCS *Sham*	24.60 + 0.74	24.75 + 0.78	23.95 + 0.74	0.520	–
PCS – Total					
LDN + tDCS	36.57 + 2.13	33.71 + 2.34	30.62 + 2.88[a]	**0.027**[c]	**2.349**
LDN + tDCS *Sham*	35.10 + 2.42	32.43 + 3.13	33.19 + 2.91	0.645	–
Placebo + tDCS	37.09 + 2.41	32.00 + 1.79	31.23 + 2.56	0.071	–
Placebo + tDCS *Sham*	37.10 + 2.12	31.75 + 2.81	30.20 + 3.41[a]	**0.032**[c]	**2.430**
PCS – Hopelessness					
LDN + tDCS	16.19 + 1.03	14.62 + 1.10	12.67 + 1.42[a]	**0.029**[c]	**2.837**
LDN + tDCS *Sham*	15.14 + 1.35	14.14 + 1.50	14.95 + 1.39	0.681	–
Placebo + tDCS	16.77 + 1.09	14.59 + 0.86	13.82 + 1.22	0.170	–
Placebo + tDCS *Sham*	16.85 + 0.99	14.05 + 1.30	12.70 + 1.62[a]	**0.003**[c]	**3.091**
PCS – Magnification					
LDN + tDCS	8.05 + 0.65	7.62 + 0.62	7.05 + 0.73	0.638	–
LDN + tDCS *Sham*	8.10 + 0.56	7.29 + 0.85	7.29 + 0.77	0.520	–
Placebo + tDCS	7.86 + 0.70	6.82 + 0.57	7.05 + 0.72	0.326	–
Placebo + tDCS *Sham*	8.10 + 0.62	6.90 + 0.81	6.45 + 0.94	0.050	–
PCS – Rumination					
LDN + tDCS	12.33 + 0.64	11.48 + 0.77	10.90 + 0.84	0.067	–
LDN + tDCS *Sham*	11.86 + 0.63	11.00 + 0.87	10.95 + 0.90	0.513	–
Placebo + tDCS	12.45 + 0.87	10.59 + 0.63	10.36 + 0.83	0.058	–
Placebo + tDCS *Sham*	12.15 + 0.77	10.80 + 0.92	11.05 + 0.92	0.348	–

FIQ, Fibromyalgia Impact Questionnaire; BDI-II, Beck Depression Inventory; STAI, State-Trait Anxiety Inventory; PCS, Pain Catastrophizing Scale.
Data presented as mean ± standard error. Friedman Test.
[a] Different from Day 1.
[b] Different from Day 21.
[c] Significant difference.

An important placebo effect was observed in the VAS, PCS, and FIQ symptoms. Corroborating, a meta-analysis performed by Migliorini et al. (2021)[23] showed an important placebo effect in patients with fibromyalgia; however, the treatment was superior to placebo in most of the studies analyzed. Another meta-analysis performed by Chen et al. (2017)[24] highlighted an improvement in pain, fatigue, sleep quality, and function in patients who received placebo when compared to those who received no treatment.

Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin that plays an important role in pain modulation by increasing the efficiency of glutamatergic synapses and decreasing the efficiency of GABAergic synapses.[25] In addition, chronic pain with high levels of sensitization is positively correlated with the dysfunction level of the descending inhibitory system of pain and with high serum levels of BDNF.[26] Patients with fibromyalgia present higher levels of serum BDNF[27] than healthy individuals, which suggests an important role of BDNF in fibromyalgia physiopathology. This study found that when the treatments were applied separately (LDN + tDCS *Sham* and placebo+tDCS), there was a decrease in serum BDNF levels. According to these results, the association may not be as efficient as when both the treatments are applied separately, since BDNF is a biomarker and not a self-reported measure.

Patients with fibromyalgia present important characteristics that directly affect its relationship with pain as well as treatment effectiveness. Patients with fibromyalgia present higher pain vigilance than patients with chronic lumbar pain as well as higher pain intensity and pain catastrophizing thought.[28] High levels of catastrophizing are correlated with more generalized pain and emotional disturbances in patients with fibromyalgia.[29] Additionally, a study showed that there is a positive correlation between catastrophizing and tender points in patients with musculoskeletal pain and fibromyalgia,[30] as well as high levels of catastrophizing are related to a low pain threshold and pain tolerance. This study found an improvement in pain-catastrophizing thoughts in patients who received LDN+tDCS and placebo + tDCS *Sham*.

Depression and fibromyalgia might be interconnected, and once serotonergic and norepinephrine drugs were used in both conditions, duloxetine (selective serotonin and norepinephrine reuptake inhibitor) and milnacipran were approved for fibromyalgia treatment in the United States.[31] This study demonstrated that both interventions are capable of reducing depressive symptoms, since LDN+tDCS, placebo +tDCS, and LDN + tDCS *Sham* improved their symptoms.

Mood disturbances, such as anxiety and depression, are among the most common psychological factors in patients with fibromyalgia, with a higher incidence in these patients than in healthy individuals.[8] Regarding anxiety, a study performed by Khedr et al. (2017)[22] reported that the related anxiety decreased using anodal tDCS in the M1 cortex when compared to tDCS *Sham*. Fregni et al. (2006)[18] reported a decrease in anxiety with active tDCS applied to the DLPFC and M1; however, a significant reduction was also found in the *Sham* group, with a similar reduction in all groups. This study demonstrated that the use of LDN associated with tDCS reduced trait anxiety in patients with fibromyalgia; however, when their anxiety-state was analyzed, only patients who received LDN + tDCS *Sham* reduced their symptoms.

The FIQ is an instrument widely used to evaluate the function of patients with fibromyalgia and is one of the most indicated questionnaires by the Outcome Measures in Rheumatoid Arthritis Clinical Trials (OMERACT) guidelines to be used in rheumatological clinical trials. In addition, the FIQ has been shown to be the most responsive self-imported improvement measurement in changes of pain intensity and total tender points and is recommended as a primary variable for fibromyalgia clinical trials. This study observed that patients who received the combination (LDN+tDCS) had a great improvement in the three domains (function, overall impact, and symptoms), and the other groups showed improvement only in the symptom domain.

This study also analyzed the adverse effects related to tDCS and found a significant prevalence of tingling, itching, and blushing in the tDCS active group compared to the *Sham* group. Overall, tDCS is a safe neuromodulation technique, even with low and transient adverse effects.[16] Regarding the adverse effects of LDN, there was a high prevalence of nausea, blurred vision, sleepiness, difficulty in concentrating, and acute mood change in patients who received LDN and placebo, which did not show a significant difference among the groups. As for limitations, this study was conducted by a large group of researchers, who may have influenced the results using different approaches. In addition, most of the variables studied were subjective (such as VAS) and within the short period of tDCS post-effect analysis.

Conclusion

The results of this study allowed us to conclude that combined LDN + tDCS has possible benefits in reducing pain frequency and intensity; however, a placebo effect was observed in pain on VAS. In addition, it was possible to conclude that it was safe and did not present severe adverse effects. Therefore, further studies need to be conducted with a sufficient methodology to supply the placebo effect. Future studies should be conducted to elucidate the effects of this association on different chronic pain conditions. In addition, further studies should be performed to evaluate the effect of this association on depression and anxiety.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

All the members of the research group who participated have contributed to this study. The Post-Graduate Program in Health and Human Sciences and the La Salle Clinic at Universidade La Salle provided materials and locations for the study.

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